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ANNALS OF TROPICAL MEDICINE  
AND PARASITOLOGY

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THE UNIVERSITY OF LIVERPOOL

ANNALS  
OF  
TROPICAL MEDICINE AND  
PARASITOLOGY

ISSUED BY THE  
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by  
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**Annals of Tropical Medicine and Parasitology**

Vol. VIII, p. 585, lines 8-10. The authors, Drs. Breinl and Young, wish to amend 'Any . . . dryness;' to read thus:—'Any lead sulphide was filtered off, washed with sulphuretted hydrogen water, dissolved in dilute nitric acid, and the excess of acid removed by repeatedly evaporating to dryness;'

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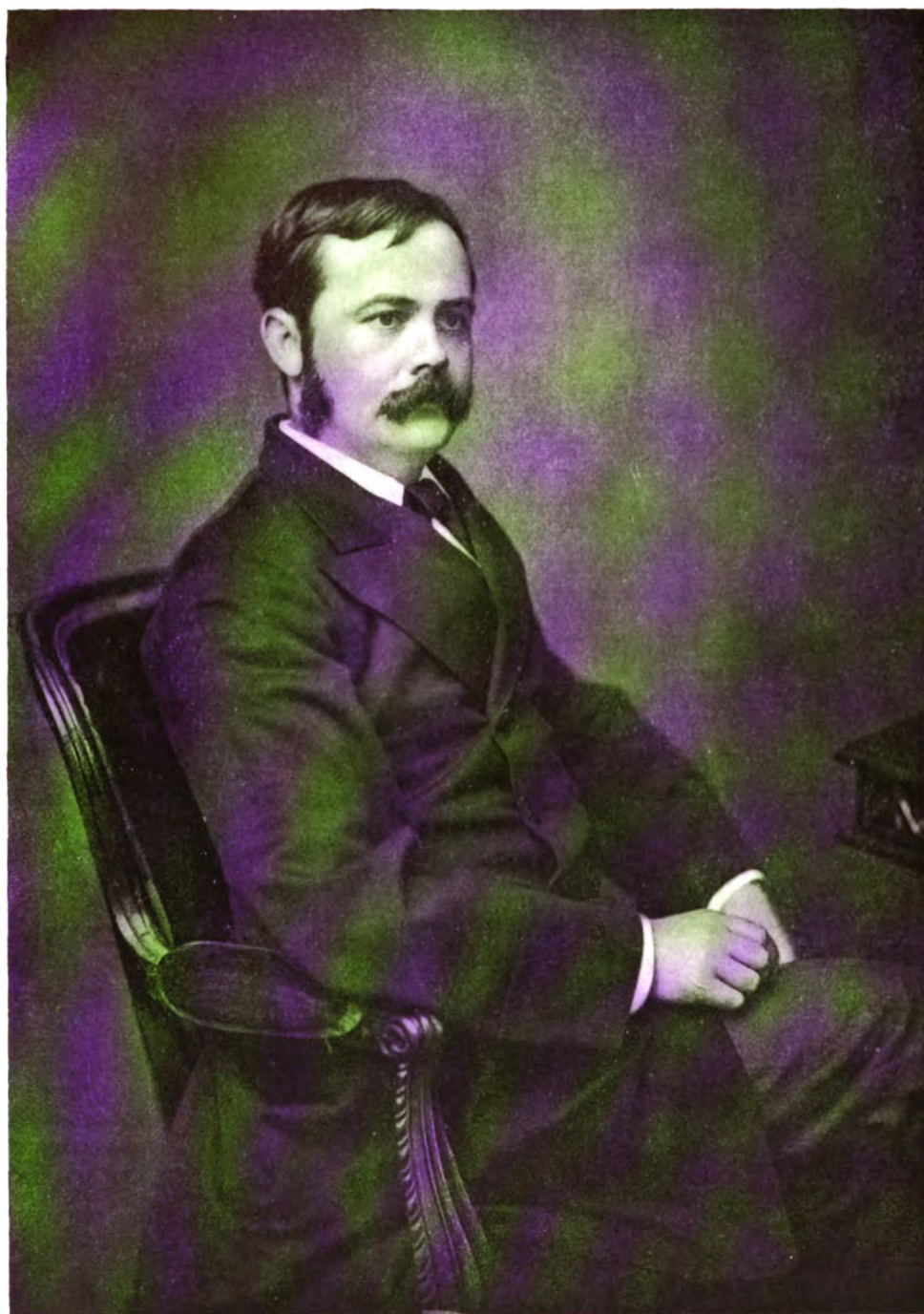
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John Everthigh  
T.H. Lewis



Volume IX

March, 1915

No. 1

ANNALS  
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TROPICAL MEDICINE AND  
PARASITOLOGY

ISSUED BY  
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Edited by  
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# LEPROSY: A PERSPECTIVE OF THE RESULTS OF EXPERIMENTAL STUDY OF THE DISEASE

BY

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AT ROBBER ISLAND

(Received for publication 18 August, 1914)

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## I. INTRODUCTION

Leprosy is the name applied to all clinical manifestations produced by an infection with the micro-organism commonly known as 'Hansen's bacillus' (*Mycobacterium leprae*).

It is a disease that has been recognised and feared for centuries, possibly already as far back as 2400 B.C., or in any case about 1300 B.C. (Ebers papyrus). Leprosy as described in Leviticus does

not, however, correspond exactly to the symptoms we know in more modern times. It should, moreover, not be forgotten that the word 'leprosy' is but an approximate rendering of the expression 'zaraath' in the original Hebrew.

Not only is leprosy the 'most ancient disease,' but it is also a widely spread, cosmopolitan scourge, which nearly rivals consumption, syphilis and cancer, if not in numbers, at least in the variety of climatic conditions under which it can flourish and increase. Though at the present day it is found, to any considerable extent, practically only in tropical and sub-tropical countries, it is known that numerous lepers existed in France, Germany, Great Britain, and other countries of Europe throughout the Middle Ages. Opinions differ whether it was imported into Europe from the East through the returning Crusaders. The disease still lingers in Turkey, Russia, the Balkan States, Norway, Spain, Portugal, parts of Italy, etc., in the form of small endemic foci. However, the ruthless individual and, in some cases, collective segregation in the thirteenth and fourteenth centuries, aided no doubt by the improving hygienic conditions of all classes, succeeded in bringing about a practically complete extinction of leprosy in Middle Europe and Great Britain. In addition to this, various plague epidemics also helped in sweeping to an early grave, in a somewhat selective fashion, the vagrant and pauper lepers sooner and quicker than individuals belonging to better situated classes.

The slow if intermittent course of the disease, the extensive mutilations and repulsive features, which are an outstanding symptom of its more advanced stages, have rendered leprosy a subject of terror and horror from time immemorial. That it is contagious has been known for centuries, but it is no doubt mainly due to aesthetic reasons that the fear it engendered was quite out of proportion to the degree in which it is communicable, under usual circumstances, from the diseased to the healthy.

Leprosy presents numerous peculiarities in its pathognomonic features. Not only is its infectivity extremely variable under slightly dissimilar or even identical circumstances, but its period of incubation varies also within very wide limits, anything between one year and twenty-five or thirty. The 'causa prima morbi' has been

known for years and years, and yet the fundamental bases of all bacteriological research, viz., artificial cultivation of the micro-organism and the experimental transmission of the disease, have presented insurmountable difficulties up to the present day. Indeed, these bases are still matters under discussion, and in any case successful results can only be achieved after numerous, repeated, prolonged, pertinacious attempts. The large numbers of bacteria which may be present in single leprotic lesions are certainly not surpassed in any other disease, with the possible exception of bubonic plague.

An excellent instance showing the influence that bacteriology exercises on clinical medicine is to be found in the effects resulting from the discovery of the 'bacillus' of leprosy by Armauer Hansen in 1872. It is true that definite knowledge of the morphology of the micro-organism was only obtained after Neisser had succeeded in demonstrating it by means of an 'acid-fast' stain in 1879. However, from the moment the bacterial origin of the disease was recognised, the communicability of leprosy became scientifically established, and all doubts in that connection could only be the outcome of an incomplete or lacking comprehension of the application of the laws of biology to practical medicine.

On the other hand the extreme difficulty encountered in transmitting the disease to animals, the repeated lack of success attending the inoculation of leprosy material into human beings (with one exception: Arning), have allowed much doubt to arise regarding the exact fashion in which leprosy is transmitted. Even the purely bacteriological observation of the fact that apparently the 'bacilli' could not be artificially cultivated, and isolated from the lesions they produced, has seriously hampered the therapeutic outlook.

Leprosy slowly and surely became the disease of negations; it could not be experimentally transmitted, its bacterium could not be isolated in artificial culture, and so to complete the list, leprosy could not be treated, let alone arrested, and no hope of cure could be entertained.

It is my intention to show whether recent experimental work allows, enables, or justifies us in taking a more active and positive view of the situation.

## II. THE CLINICAL NOSOLOGY OF LEPROSY

Two clinical types of the disease are generally distinguished and no more. Such views are partly owing to the influence of Hansen, who considered that it was not necessary to make further sub-divisions because practically all lepers presented features at one time or another of their existence (that is, if they lived long enough) which allowed them to be classed either as cases of (i) nodular (hypertrophic) leprosy, or (ii) maculo-anaesthetic, otherwise called atrophic, smooth leprosy.

From a purely clinical point of view this classification holds good, however, only within certain limits. It is a commonly accepted rule of nosology that bedside or clinical nomenclature does not take into consideration so much the eventual course of a disease, as its etiology and the symptoms and appearances which, however fleeting and changeable, can be brought under a definite scheme of relative incidental constancy. The classification used in relation to recognised clinical types of tuberculosis, syphilis, and other complaints, which often run a chronic course and vary largely in their symptomatology, seems clearly to show which are the determining factors ruling the designation of clinical entities.

It is no doubt quite correct, from a general point of view, to state, as Hansen did, that a 'mixed' leper (i.e., a patient who from the very beginning shows features of both the nodular and maculo-anaesthetic type) who lives long enough and undergoes a natural process of elimination of the great majority of the acid-fast 'bacilli' from his lesions, will eventually be classifiable as a purely anaesthetic leper. Many of these instances of 'lepra mixta,' however, do not reach this ultimate stage, because death steps in before completion of the process of evolution into a different type, and in any case the primary condition may last for many years.

Without unnecessarily labouring this point any further, I believe that there is quite sufficient justification in considering every single case of leprosy as classifiable on the basis of the clinical and bacterioscopic features at the time of examination, irrespective of the expected course of the disease later on. In other words, the clinical diagnosis is to be considered of a temporary nature, as far as the naked eye appearances (and to a certain extent also the bacterioscopic results) are concerned. The diagnosis leprosy is the



only unchangeable feature, though it may need modification by the adjectives 'arrested' or possibly even 'cured.'

Leaving aside sub-divisions founded on partial or purely localised symptoms, I believe that on examination of any great number of lepers, in any part of the world, more or less numerous specimens of the following varieties or even distinct types of the disease may be distinguished.

1. Nodular type with more or less marked or diffuse tuberosities which generally are most developed on the face, but of course can also affect any other part of the body, including (though somewhat rarely) the scalp, the soles of the feet, the tongue, and the palms of the hands. Leprous nodules should contain numerous acid-fast micro-organisms, and in certain instances may be located as loose or movable small lumps which can be shelled out from under the skin.

Nodular leprosy may give rise to visceral lesions, which are remarkable for the enormous quantity of acid-fast 'bacilli' that can be detected accumulated in the tissues of the spleen, liver, testicles, lymphatic glands and lungs.

2. Maculo-anaesthetic type with more or less numerous spots which have a slight predilection for the trunk (chest, back, buttocks) but are certainly also very frequently present on the limbs. The spots in advanced cases may show a greater or lesser amount of anaesthesia. It should be noted that often the anaesthesia is definitely localized in portions not showing any maculae. In many cases, however, the anaesthesia is by no means complete, and should more correctly be termed paraesthesia. The patients cannot distinguish hot and cold, sharp or blunt in the affected areas, but are still sensitive to touch. In early stages the maculae may present a ringed appearance; the centre then may be normal in relation to sense of touch, etc., whilst the outer ring, if anything, is slightly hyperaesthetic.

The maculae appear to be the result of the localisation (and possibly disintegration) of a small number of casual micro-organisms in the skin. The anaesthetic areas are due to the 'bacilli' invading the sheath of the nerves, causing a proliferation of connective tissue, which brings about a slow degeneration of the corresponding nerve fibres. In advanced cases the bacilli invade

the ganglia of the spinal cord, hence the practical impossibility, under certain circumstances, of distinguishing leprosy clinically from syringomyelia.

3. Mixed or combined leprosy, that is, cases where from the very earliest noticeable onset of the disease, and for years afterwards, definite areas of paraesthesia (insensibility to heat and cold, incapacity of distinguishing blunt from sharp) and anaesthesia can be made out, accompanied by maculae and small nodules teeming with acid-fast bacteria.

An excellent description of this type has been given by Glück, who called it 'lepra tubero-anaesthetica.'

These three main types can be sub-divided into the following relatively rare varieties and singular forms:

1. Lupoid or serpiginous leprosy. Among the 2,000 lepers I have had under observation in South Africa I have only met with the disease three times. Another instance of the variety was seen in London. It is recognisable as leprosy by the presence of nerve-lesions, but acid-fast micro-organisms are very scarce indeed in any of the superficial patches. The expression 'patches' is used because the skin-lesions are much more raised and thickened than mere 'maculae,' and yet do not deserve the appellation of nodules, being too flat and extensive. They spread by forming peculiar confluent configurations, which can be best recognised by referring to Pl. V, fig. 1.

The patches are a peculiar coppery colour, and do not show any marked tendency to break down and ulcerate, which in addition to other peculiarities distinguishes them from lupus.

At times single patches whose colour, texture and general appearance corresponds closely to that of these serpiginous lesions, are noticeable in cases of leprosy, whose remaining clinical features differ in no other fashion from the usual nodular type.

In other instances the patches may not be confluent and serpiginous, but are distributed unevenly all over the body, varying in size from the diameter of a threepenny-bit to the extent of a man's hand or more. They may appear relatively rapidly, within a fortnight or so, and are accompanied by localised paraesthesia. In such case the diagnosis usually presents considerable difficulty, because acid-fast bacteria may be practically absent in the lesions and nasal mucosa.

Jadassohn has described somewhat similar varieties of leprosy as the 'tuberkulid' type, in view of the resemblance which microscopical specimens of the lesions bear to tuberculosis of the skin.

2. Many cases of maculo-anaesthetic leprosy begin with paraesthesia, numbness, and a peculiar 'leathery' feeling, usually of the ring and little finger of one hand, or tingling sensations with small areas of definite anaesthesia may be present in a hand or foot. Such conditions can last for a considerable time, as much as three or more years, before definite maculae make their appearance. Some cases, after a prolonged premonitory stage of this nature, may develop nodules and finish as definite hypertrophic leprosy.

Maculo-anaesthetic lepers may heal in such a fashion as to allow hardly any traces of their spots being visible to the naked eye; the trained observer may just be able to make out a simple configurated discoloration or slight livido. Needless to say, definite areas of anaesthesia are usually easily made out in these patients. As long as these conditions last it appears quite correct to classify them as simple paraesthetic or anaesthetic leprosy.

3. Under certain circumstances maculae or spots may appear before any nerve symptoms are traceable. Even to the experienced clinician the leprotic nature of the spots may appear doubtful, other conclusive symptoms being absent and possibly no history of contact being available, though the latter has but a relative value. In these rare occasions only prolonged observation, aided and controlled by the microscope, can afford us certainty in diagnosis. Eventually, in the course of time, a definite maculo-anaesthetic stage is reached, but during this initial period the condition is one of simple macular leprosy.

When dealing with dark-skinned races great care must be taken not to mistake for macular leprosy one of the numerous tineae which have been so ably classified and investigated by Castellani.

4. In some instances leprotic symptoms are seen which are somewhat too prominent to deserve still the name of 'maculae' or spots. The edges are considerably more raised than is usually the case in smooth leprous lesions, owing to active proliferation of the superficial dermal tissue. The periphery is studded with numerous minute papulae, which have a somewhat vesicular appearance. The central portion may or may not participate in this process of granulation, but a certain degree of desquamation is generally

present. In any case the appearance of the inner area is angrier and more 'active-looking' than what is seen in the usual run of maculae, though parts of the surface do not usually participate to such a marked degree in the process of granulation and eruption noticeable at the margin.

It may be objected that practically all maculae of lepers go through periods of increased activity or exacerbation, during which they may show some of the appearances described. However, the case illustrated in Pl. V, fig. 2, has remained in this condition for over three years, and moreover, the localisation of the lesion is somewhat peculiar. In addition, the maculae of many lepers never reach the condition of marginate proliferation shown in the photograph.

Of course this whole condition is but the result of an intensified tissue reaction, such as generally takes place to a much lesser degree in most maculae. The diagnosis, however, may still present difficulties, because, as in the instance illustrated, other definite leprotic symptoms, such as anaesthesia, may be absent or cannot be detected at the time of examination. I should like to draw attention to the existence of these intensified 'maculae' without insisting on their separate classification.

5. Diffuse or hyperaemic leprosy is also scarcely a separate variety, but simply an initial or premonitory stage of nodular leprosy. It consists in a peculiar diffuse, puffy swelling of the eyebrows, the lobes of the ears and the alae nasi, which in addition takes on a peculiar pinkish-bluish hue. This condition may last for years and imperceptibly merge into the well-known condition of leprosy generally known as 'facies leonina.' In rare instances the disease may not make further progress, but eventually improves and leaves, as the only trace, a peculiar wrinkled and dry appearance of the skin of the face. I have so far not found any of such arrested cases without smaller or larger areas of anaesthesia.

These three types and five varieties or peculiar diversities from the usual clinical appearances of leprosy appear to me to cover all forms of leprosy, as far as I have been able to observe them. In addition, there are numerous combinations which can result through atypical and transitory appearances of the disease mixing in varying proportions.

Bullae, ulcers, suppurations and necrosis may considerably alter and add complexity to the clinical features of the symptomatology of leprosy. They should, however, be considered as complications of the primary infection; moreover, they are too variable and inconstant to allow definite systematization.

Careful description of the majority of these symptoms is also given by Abraham (1910) where special stress is laid on the fact, and rightly too, that the diagnosis is in many cases but the description of a stage of the disease.

These various sub-divisions have not been made to complicate further a diagnosis which under usual circumstances is already sufficiently difficult, but on the contrary to induce a careful observation of the various phenomena of a disease whose correct, exact, and above all early recognition is at all times a matter of considerable importance.

On two occasions I have noticed in lepers peculiar psoriasis-like maculae, but in these instances the possibility of psoriasis and leprosy occurring together could not be excluded.

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#### III. MICROSCOPICAL FEATURES OF LEPROUS LESIONS

The minute anatomy of leprotic lesions or histo-pathology of leprosy is the study, by means of the microscope, of the relationship or direct and indirect action of the lepra-bacterium to and on the tissues it has invaded. Accordingly, every investigation or analysis of the appearances detected by the microscope must take into consideration not only the presence or absence of acid-fast 'bacilli,' but also, in the former eventuality, the quantity and localisation of the 'bacilli' discernible in the sections being examined. Accordingly it is to be understood that the microscopical diagnosis of a leprous lesion is bacterioscopic and histo-analytic.

Good descriptions of the microscopical appearances of leprosy have been given by Lie, MacLeod, Jadassohn and others, and an even prolonged observation of the material available in South Africa does not bring to light any new facts of fundamental importance, except to show once more the necessity of clearly distinguishing between leprosy and tuberculosis by means of animal experiments and any other methods available for the purpose. In referring to the lesions found in the spleens of lepers, MacLeod (1910) expresses his view as follows (page 314):

'In the spleen, in addition to leprosy infiltrations in the trabeculae, pulp and malpighian corpuscles, extensive necrotic masses have been described by Arning (Schäffer, *Leprosy*, 1900, I, p. 11) like those of tuberculosis with typical Langhans giant cells. Such cases have to be further verified from the point of view of tuberculosis before they can be finally accepted.'

In several autopsies (5) at Robben Island I have found in the spleen of nodular leprosy lesions similar to, if not identical with, those described and illustrated by Schäffer. The inoculation experiments into guinea-pigs were, however, positive; that is, the animals experimented upon died within two to four months with extensive generalized lesions of a tuberculous nature.

These investigations are by no means concluded, and they have the limited value of single instances. They simply show that 'tuberculous' lesions in a leper are not necessarily always caused by Hansen's 'bacillus' alone.

The uncompromising standpoint of Hansen, that leprosy and tuberculosis were so absolutely distinct from each other that no single common feature was thinkable, is no more defensible or convenient, not even from a didactic point of view. At the present day we have learnt through the more intimate knowledge of many maladies and the properties of numerous pathogenic micro-organisms, that dissimilar diseases can be caused by microbes which are practically indistinguishable, and that on the other hand analogous pathological conditions can be brought about by micro-organisms which behave in a totally different fashion in artificial culture and laboratory tests. As cases in point it is only necessary to mention at random yaws and syphilis; kala-azar, infantile splenomegaly and oriental sore; typhoid and paratyphoid; human

and bovine tuberculosis, plague and pseudo-tuberculosis. Pathology has learnt the truth of Goethe's dictum 'Alles ist Ubergang.' The increasing exactitude of clinical methods of observation ought to enable us to avoid the pitfall of considering two allied diseases as identical, since we understand that hard and fast types are only extant for the purpose of classification.

For some time discussion has been rife whether leprotic tissue ever contained giant cells of the Langhans type. It is now practically unanimously admitted by those who have had opportunity of seeing a sufficient number of slides from different cases, that giant cells do occur in typical leprotic tissues, for they have been observed by Thoma, Jadassohn, Ramon y Cajal, Rikli, Schaeffer, Dohi, Babes, Kedrowsky, Matsuda, Gurd, Bayon and G. W. Robertson (verbal communication). Even Lie, who originally held that giant cells were only present in leprosy when the disease was complicated by tuberculosis, now admits their presence, with the reservation that they are not morphologically absolutely identical to the Langhans type found in tuberculosis (verbal communication).

In discussing the nosology of leprosy, stress was laid on the temporary nature of many conditions in leprosy, as a result of the extremely chronic course of the disease and the facility with which it can be complicated by other infections. In addition we have to consider the multifarious appearances of the disease *per se*. These factors influence also the histological features to a considerable extent, so that it is not possible to state, except in a somewhat general fashion, which are the microscopical features that render the diagnosis 'leprosy' certain in any section. No single element is of decisive value, except the presence of numerous acid-fast rods, and this even with several qualifications. Hansen's 'bacillus' is generally only to be found in large numbers in advanced nodules and nerve-lesions, and is practically absent from maculae and leprotic papulae, that is, only to be detected after a very prolonged search and by special methods.

Unna's definition of a leproma is as follows:—

' . . . . a diffuse granuloma, whose peculiarity consists, on the one hand, in its limitation to the connective tissue elements, and specially to the lymphatic system of the skin, and on the other in the enormous growth of organisms, whose number far exceeds

anything we are accustomed to find in other infectious diseases. . . . To the paucity of the cellular elements and the preponderance of the organisms dormant in the bacillary mucus, the remarkable indolence and relative benignancy of these growths may be ascribed.' (Unna-Walker; *The Histopathology of the Diseases of the Skin*, 1896, p. 616.)

Jadassohn sums up his investigations on the subject in the following words\* (l.c. p. 871):—

'The leprosy bacillus produces a non-characteristic type of inflammation, attended by the formation of granulomatous tissues with different features of a degenerative character, which can even deserve to be classified as necrosis; this may in some instances take on a tuberculous appearance, signalised by sclerosis, and direct or indirect destruction (Untergang) of various specific parenchymata. As a rule the leprosy bacillus causes only an indolent reaction and the resistance displayed by tissue against its luxuriant growth (Wucherung) and toxins is quite remarkable. Deposits of bacilli may be detected even in tissue which otherwise appears to be quite normal, and in cells which cannot be shown to be altered in any known fashion. The histological features are also very variable as a result of the diversity of clinical types.'

'The typical leproma, wherever it may be situated in the body, has the broad characteristics of other infective granulomata, with certain peculiarities which render its diagnosis possible' (MacLeod).

It is evident from what has been said above that distinctive features need not necessarily be present in many lesions which from their occurrence and localisation we know to be definitely leprotic. By avoiding generalisation, and considering single types and anatomical situations by themselves, it is, however, possible to recognise certain definite, recurring, important features.

*Skin. Nodules.* Numerous acid-fast rods occur, especially in matted groups, there is absence of advanced necrosis (except in the presence of open sores), and no caseation or giant cells with a necrotic centre. Giant cells containing single acid-fast 'bacilli' may, however, be present, but generally they are not numerous, and show fewer peripheral nuclei than is the case in tuberculosis. The 'bacilli' may be scattered loosely in the parenchyma of the nodule

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\* Translated by the Author of this paper.



or may have invaded various cells of endothelial and fibroblastic origin, in which case it is especially remarkable that the structure of the protoplasm and nucleus or even the morphology of the bacterial acid-fast rods has not suffered in any noticeable fashion. Plasma cells are rare as opposed to yaws and syphilis, where they are especially numerous.

A peculiar narrow band of hyaline, glassy tissue, containing no 'bacilli,' is often noticeable just under the epidermis.

Acid-fast bacteria may be present in such quantities that in stained sections (Ziehl-Nielsen) the whole tissue appears a dull red to the naked eye. In other instances 'bacilli,' though very numerous, are distributed singly or by twos and threes, and can only be detected with high-power lenses.

*Skin. Patches.* Acid-fast bacteria are not numerous. They can only be detected with great difficulty. They are not bunched or massed, but present in little groups of few 'bacilli,' which may apparently be situated extra-cellularly or they may have invaded cells without causing any alteration in the structure of either protoplasm or nucleus.

Giant cells with numerous peripheral nuclei are often present. At times a peculiar coagulation or discrete necrosis of the tissue may be noted in parts, which shows hardly any definite structure. It has a filamentary appearance and stains easily with eosin. Such small areas of degeneration are circumscribed and show one or two giant-cells at the periphery.

The vessels and capillaries can be seen partaking in a process of proliferation, accompanied by an increase in the number of endothelial cells forming their walls.

The epidermis is often found thinner than usual, and the epithelial villi are somewhat flattened out, or the epithelium may appear as a border of uniform breadth.

Spindle-shaped cells (young connective tissue cells) may be numerous, and plasma cells are not infrequent.

*Skin. Maculae.* Nodules and patches partake of the nature of infectious granulomata. The macula belongs, however, to another pathological group, which can be compared with a condition of slight chronic irritation, possibly not very dissimilar to that seen in Erythema perstans.

Various degrees of perivascular cellular deposit correspond to the different intensity of eruption in single maculae, but, as a rule, a slight infiltration of spindle and round cells can be detected surrounding the capillaries and sweat-glands. The hair-follicles may also undergo a similar process of localised infiltration and proliferation. Giant cells may be present, but hardly any acid-fast bacilli, unless nodules are to be found in other parts of the body.

The histological appearances I have described agree with the view that maculae are a local anaphylactic symptom, caused by the toxic products set free by the lepra-bacilli, after disintegration induced through the agency of the defensive properties of the tissues involved. This process takes place, however, when only few germs are present, because lepra 'bacilli' in common with other acid-fast micro-organisms are very resistant.

Regarding the changes occurring in the peripheral nervous system, the suggestion has been made by Lie that 'bacilli' are only present in maculae at the very beginning, and that later they disappear into the nerve-endings and finally ascend along the peripheral nerves. It can, however, be objected that in many cases anaesthesia appears to set in without the primary production of maculae in the corresponding area, and that many maculae do not show any definite anaesthetic symptoms, though they have lasted for years.

The other hypothesis considers the neuritis to be a descending process (a contrast to the 'ascending' process put forward by Lie), and accordingly the result of deposit following generalised infection. In any case, though the nerves may be involved in any single type of leprosy, it is in the maculo-anaesthetic type that this invasion and localisation gives rise to the most striking and apparently relatively rapid results.

In thickened peripheral nerves, under certain circumstances, numerous 'bacilli' may be found, as in skin lesions. Generally they are situated in massed groups in the spindle-cells of the perineurium, or loose in the interstitial lymphatic spaces; at times they may be distributed with a certain regularity in the cells of the endoneurium, so that they appear to be in rows rather than in clumps. In the course of time they may get eliminated and leave behind a marked thickening, consisting of connective tissue with a

few stray degenerated and fragmented nerve fibres, in which the general disposition of a nerve-bundle is still recognisable.

Practically every organ of the body has been found to be the site of leprotic lesions by some observer or other, but deposits of lepra-bacilli are more usually found in the lungs, liver, spleen and testes. In the lungs the frequent complication with tuberculosis is very disconcerting, and in many instances a decision as to the pathological process involved can only be arrived at by tests with inoculated guinea-pigs, though it is quite possible that even this crucial experiment may not have an absolutely decisive value. Babes gave considerable attention to the question of the lesions found in the lungs of lepers, and has come to the following conclusions which well deserve to be quoted in full:

- (1) The lungs are normal, and do not contain lepra bacilli.
- (2) They appear healthy to the naked eye and under the microscope, but notwithstanding contain leprosy micro-organisms.
- (3) They show the features of hypostatic pneumonia or broncho-pneumonia, without any leprosy micro-organisms being recognisable, whilst pneumococci and streptococci are present.
- (4) They may show signs of tubercular localisation with excavations, caseous pneumonia and peribronchial nodules, tubercle bacilli and without the leprosy micro-organisms.
- (5) In one single case he found caseous degeneration without any tubercle or leprosy bacilli or other bacteria.
- (6) In cases of chronic interstitial pneumonia peribronchial foci of a leprosy nature were noticed.
- (7) The lungs may be the seat of extensive desquamative or fibrinous localisation of a leprosy nature, with countless lepra bacilli, which may be accompanied by the formation of vomicae.
- (8) Gangrenous bronchial cavities, and bronchial affections of a leprosy nature, were found in the centre of necrotic foci or surrounded by an interstitial or desquamative pneumonia.
- (9) Cases exist where leprosy and tuberculous lesions combine, and accordingly are very difficult to diagnose correctly.

The lesions of leprosy lungs are very similar to those of tubercle, but it must be remarked that leprosy runs a more chronic course, the resulting tissues are more solid, caseate less frequently, and are less prone to be destroyed or eliminated. When considered from this standpoint leprosy lesions in the lungs resemble the late manifestations of syphilis of the lungs.

We are thus enabled to distinguish not only a chronic diffuse interstitial or trabecular form of leprosy of the lungs and a sclerotic nodular form, but also an acute, and at times caseating, parenchymatous variety of the disease. In the latter we also find fibrinous-pneumonic catarrhal lesions.

In all cases he succeeded in proving, by means of cultures, and in some cases also by microscopical examination in the bronchial forms, the presence of diphtheroids in company with the lepra bacilli. These organisms are associated at times and in varying manner with bacilli of the coli group; at other times with filamentary, branching micro-organisms or with streptococci or pneumococci in the acute pneumonic or caseating varieties ('Lepra; Handbuch der pathogenen Micro-organismen' of Kolle-Wassermann, 1906, Ergänzungsband, pp. 174-175).'

Leprotic lesions of the other organs may be accompanied by a greater or lesser degree of amyloid degeneration. Amyloid of the spleen, liver, kidneys, may be present in lepers who die with the features of anaesthetic cases, but typical leprotic lesions of the inner organs are generally only found in nodular cases.

*Liver.* The most frequent change noted is a peculiar interstitial infiltration, accompanied by numerous acid-fast rods situated in 'lepra-cells,' that is, mononucleated round cells whose protoplasm is swollen out and riddled with vacuoles. The liver cells may also contain 'bacilli,' though this is not frequent. Generally the parenchymatous cells are but slightly altered, except for the presence of lardaceous disease, and under certain circumstances may contain a deposit of brownish-yellow pigment. The capillaries appear dilated and may also contain lepra-micro-organisms, which may have invaded the endothelium or lie loosely in the lumen.

In common with the other organs it is often very difficult to decide in sections of the liver whether we are dealing with simple deposits of 'bacilli' which have been carried by the blood stream and have accumulated passively in the walls of the capillaries, or whether we are dealing with a chronic but progressive pathological process involving multiplication of the germs and an ever increasing local spread of the leprous lesions.

In single cases giant cells and necrosis were detected, but I fear that in these instances a complicating tuberculosis could not be excluded. The same applies to the similar alterations seen in the spleen.

*Spleen.* In the spleen the acid-fast bacteria can be so numerous that their presence can be detected by the naked eye on staining. They may be scattered about in small clumps in the pulp, especially in the neighbourhood of the capillaries. The presence of lepra-cells may be under certain circumstances a noticeable feature of the lesion. Though a spleen may be in an advanced

stage of leprotic invasion the resulting enlargement may be hardly noticeable.

*Testicles.* The connective tissue between the tubuli is seen to have proliferated actively, and to contain more or less numerous acid-fast rods, some lying loosely, others enclosed in lepra-cells and in the endothelium of capillaries.

Numerous degrees of connective tissue proliferation can be found in the testicles from different patients, and under certain circumstances even from different parts of the same testicle. The bacilli present also vary very much in numbers—from few bacilli, which can only be detected by means of an oil-immersion lens, to deposits rivalling in numbers those seen in the nodules of some lepers.

*Lymphatic glands.* There is no doubt that the bronchial, mesenteric, and other lymphatic glands can be affected by a pure leprous infiltration, which microscopically shows very numerous acid-fast rods enclosed in cells of different sizes, some with hypertrophied protoplasm containing numerous vacuoles. Lymphatic glands may also be the seat of a mixed infection with tuberculosis and show necrosis and caseation.

From this brief survey it will be seen that in dealing with the microscopical diagnosis of leprotic lesions we are continually faced with the difficulty of distinguishing lepra from tuberculosis, and that in many instances the decision must be left with the results of inoculation into guinea-pigs.

It should not be forgotten that leprosy is a slower and more chronic complaint than even tuberculosis, and that in certain instances the possibility is given that a tubercle which has spontaneously healed may be invaded by Hansen's 'bacillus,' in which case the crucial experiment on animals would give a negative result, though the histological lesions might show a definite tuberculous character.

With the exception of cases where the presence of numerous giant cells of the Langhans type, or very similar in appearance to those seen in tuberculosis, coupled with necrosis or caseation, render the presence of a mixed infection with tubercle probable, the main microscopical features of a leprotic lesion can be summed up in the following words:

Very numerous acid-fast micro-organisms, singly or in matted

clumps, lie loose in the tissues and lymphatics or wedged in compact masses in interstitial spaces. Similar acid-fast bacteria may be present in varying numbers in the protoplasm of various cells, in which under certain circumstances vacuoles can be detected. The endothelial cells of the lymphatics may also show a marked degree of bacterial invasion. The tissue reaction and production of connective tissue is relatively insignificant in all instances, and the resulting degeneration or necrosis of the tissues affected is quite minimal in proportion to the number of germs present in the lesions.

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#### IV. MORPHOLOGY AND STAINING PROPERTIES OF HANSEN'S 'BACILLUS'

The *Mycobacterium leprae* belongs to a group of acid-fast bacteria which in morphology and staining reactions are very similar to the *Mycobacterium tuberculosis*. The group may be considered to consist of the micro-organisms causing spontaneous tuberculous disease in human beings, cattle, horses, pigs, birds, etc. All these bacteria are relatively easily isolated on artificial media, at body heat, from the lesions in warm-blooded animals, with morphological and tinctorial properties analogous to those which may be seen in infected tissues. A sub-division of this group embraces several acid-fast germs, which are generally present in diseased tissues in enormous quantities and are extremely difficult to isolate in artificial culture. This sub-division may be considered to include the *Mycobacterium leprae*, the *M. leprae rodentium* (rat leprosy 'bacillus'), and the micro-organism of Johne's disease in cattle, and that causing the analogous disease of sheep mentioned by Twort and Ingram, Vukovic, McGowan. It is quite evident

that a classification based on such principles cannot be satisfactory from a bacteriological standpoint, but it is simply brought forward to show once more that the leprosy 'bacillus' does not stand absolutely alone in many of its peculiarities.

Lehmann and Neumann's (1912) definition of the group *Mycobacterium* is the following (p. 582):

'Thin, slender rods, often showing typical dichotomous branching, at times giving rise to filaments, with or without branching. If stained with warm carbol-fuchsin, the colouring matter cannot be extracted by acids, that is to say, the bacteria are acid-fast and behave towards stains somewhat like the spores of common fission-fungi. In some species the acid-fast properties are but slightly developed; in fact, may even be absent.'

According to Lehmann and Neumann's definition, the mycobacteria are not true 'bacilli,' and the possibility of micro-organisms of the group showing true branching is recognised, as also the fact that acid-fastness is a property which varies in different bacteria belonging to the group. These admissions are important in view of the fact that lately some bacteriologists have denied both conclusions when applied to the microbe of leprosy.

A satisfactory classification of Hansen's 'bacillus' is not a matter of purely theoretical interest. It has definite practical importance, because we have reason to expect that an exact knowledge of its bionomics will help isolation by means of artificial culture, and in such a fashion clear up the numerous obscure points in connection with the epidemiology and therapeutics of leprosy.

The irregular morphology of the micro-organism of leprosy tends to confirm the view that it is not a true 'bacillus,' because of the lack of constancy in shape, thickness and length, which in addition to various other features connected with the presence of endogenous spores, flagella, etc., determines the diagnosis 'bacillus.' The lepra bacterium is markedly pleomorphic, that is to say, in smears from nodules single germs can be detected which vary in thickness between  $0.3 \mu$  and  $0.5 \mu$ , and whose length may be anything between  $1 \mu$  and  $5 \mu$  and in rarer instances even  $6 \mu$  or  $7 \mu$ .

Hansen's 'bacillus' is often seen to contain small, fine granules (Babes-Ernst) which are not as acid-proof as the rest of the

bacterium. At times, peculiar hyaline breaks can be detected in the rods; they have been considered to be spores.

Another peculiarity of the bacillus is its breaking up into small coccoid bodies, which Deycke considered to be a sign of degeneration, but which the observations of Martinez-Santamaria seem to indicate are possibly developmental stages prior to multiplication.

The reasons for which it is possible to classify Hansen's 'bacillus' as the acid-fast stage of a *Mycobacterium* closely allied to the *Actinomyces* are based on morphological grounds and deductions by analogy, in addition to the interpretation of cultural attempts.

The morphology of Hansen's 'bacillus,' when examined with high-power lenses (say 2 mm. apochromatic, comp. eyepiece No. 12) and in sufficiently thin smears, can be seen to be exceptionally variable; structures with one end tapering and the other definitely thickened (clubbed) are mixed with rods of uniform thickness but of different lengths and fragments, which seem to consist of a series or chain of small beadlike coccoid bacteria.

At times in smears taken from nodules branching forms are seen; they are, however, rare. These peculiar pleomorphic appearances, especially the clubbed and branching forms, are typical of the fragments of actinomycotic germs having the tendency to break up into acid-fast fragments. The variations in morphology have lately been commented upon by Galli-Valerio, who, in discussing the relationship of the genera *Corynebacterium*, *Mycobacterium* and *Actinomyces*, has not failed to note the numerous points of contact between these groups.

From the standpoint of analogy we know from the observations of Petrone (1884), Metschnikoff (1888), Fischel (1892), Babes and Levaditi (1897), Schulze (1899), Lubarsch (1899), Lehmann and Neumann (1912), Galli-Valerio (1912), Foulerton, and others, that true branching can occur in *Mycobacterium tuberculosis* and that its appearances in sputum and tissues of experimental animals are at times distinctly like those brought about by actinomycotic germs, especially in the production of radiary deposits of bacteria. Abbot and Gildersleeve have noted similar appearances in connection with 'saprophytic' micro-organisms. Further, observations of Silberschmidt (1899), Galli-Valerio (1910), Birt and Leishman, and



others, have made us acquainted with a group of germs which clearly can be classed with the Actinomyces or Streptothrix class, yet in artificial cultures and on injection into animals show numerous acid-fast fragments very similar to *M. tuberculosis*.

That an acid-fast rod seen in tissues, can be found to be a filamentary branching micro-organism in artificial cultures, is an observation which has been made so repeatedly by those working with *A. caprae*, Birt and Leishman's Streptothrix, that there is no necessity to dilate further on the subject.

Accordingly we may deduce that it is by no means far-fetched to suggest that Hansen's 'bacillus' may present a filamentary, acid-labile appearance in artificial culture, though the *M. tuberculosis* is consistently acid-fast on the usual laboratory media: and even in this instance Frei and Pokschischewsky, and later Wherry, have shown that under certain circumstances this property can be made to vary.

The interpretation of the cultural results of investigators whose bacteriological technique was not open to criticism (Beauchamp Williams) shows us that in the great majority of cases their culture tubes have either remained sterile for months and months (Fraser and Fletcher), or that their repeated efforts were only rewarded by the isolation of a singularly pleomorphic diphtheroid with slight acid-resisting properties, that is to say, that after prolonged staining with carbol-fuchsine it could be, and was, only partially bleached by a weak acid such as one per cent. sulphuric. This diphtheroid was usually found to have peculiar filamentary forms.

The relationship of the genus Corynebacteria to the Actinomyces is also very close; to give an example out of a numerous series we have the *Corynebacterium necrophorum* (Lehman and Neumann) which Schmorl considers as a Streptothrix and other authors as an Actinomyces.

As a matter of fact, the bacteriological literature of the last ten years contains numerous, indeed very numerous, hints that a strict separation of bacteria on cultural characteristics alone is extremely difficult, to say the very least, because of the existence of very many micro-organisms which do not conform in every case with any single distinctive test or feature.

It will be seen that, though definite reasons are extant to

postulate that Hansen's 'bacillus' belongs in reality to the Actinomyces, Actinobacteria or ray-fungus group, still the last conclusive test is a matter of surmounting the difficulties inherent to the artificial isolation and cultivation of this bacterium.

#### STAINING PROPERTIES OF HANSEN'S 'BACILLUS.'

In a recently published study on the bacteriology of human and rat leprosy Wolbach and Honeij (1914) come to the conclusion that :

'The possibility that the non-acid-fast and acid-fast organisms isolated from cases of leprosy are related and may be converted one into the other is one we must take, however reluctantly, into consideration, because of the great length of time the leprosy bacillus resides in the human body and the variety of conditions it is subjected to by virtue of the stage of the lesion and anatomical situation. The loss and acquisition of properties as distinctive as that of acid-resistance are well known in the field of bacteriology and indeed the changes undergone by the tubercle bacillus towards staining reactions is the strongest claim for the possibility.

Very numerous methods have been published with the purpose of communicating a strictly specific stain for the leprosy micro-organism, that is, a stain which would show up in some distinctive colour Hansen's 'bacillus,' whilst all other bacteria, however similar but not identical, would not take the same hue. The majority of these methods are based on the property of the *M. leprae* of taking up carbol-fuchsin or a similar stain more readily than Koch's 'bacillus,' and, moreover, on the greater resistance, as a rule, of the latter to the bleaching properties of mineral acids or strong alkalis.

Accordingly it will be seen that we are dealing with differences in degree and not in quality, and as to be expected, such methods of differentiation are apt to fail in a fair proportion of cases and be unreliable in the remaining instances.

Our experience in dealing with the sections and smears in the laboratory at Robben Island is that to obtain well stained leprosy bacilli in sections it is advisable to use  $2\frac{1}{2}$  per cent. carbolic in making up the fuchsin solution, to stain in the warm solution one or two hours according to thickness of section, and to differentiate with one per cent. hydrochloric acid in the common methylated spirit of commerce. Counterstain with alum-haematoxylin.

Skin fragments are always fixed in absolute alcohol, specimens from organs in 4 per cent. formaldehyde. Embedding in paraffin is advisable, though excellent sections can also be got in celloidin.

For the quick staining of nasal smears, etc., Pappenheim's carbol-fuchsine-methylene-blue-coralline method gives reliable results.

Unna has described a method of distinguishing lepra 'bacilli' which have been fixed alive from those that were brought dead into the fluid. The validity of this distinction is, however, only founded on circumstantial evidence.

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### V. ARTIFICIAL CULTIVATION OF THE MICRO-ORGANISM OF LEPROSY

The first attempts to cultivate Hansen's "bacillus" were made by Sir Patrick Manson many years ago, at a time when aniline dyes had not yet been introduced into bacteriological technique. Later on, in 1882, Hansen thought he had succeeded in getting the "bacillus" to grow artificially in some serum contained between a microscope slide and slip, but he never followed the matter any further after mentioning this observation.

Neisser in 1886 considered that multiplication of "bacilli" from a nodule had taken place on cooked egg and blood serum. In any case he was not able to get further generations to grow.

Bordoni-Uffreduzzi in 1887 cultivated from the bone marrow of a case of leprosy a partially acid-fast or acid-resistant micro-organism, which, from the description given and the specimens seen, I would consider to be morphologically a diphtheroid. It was not quite as acid-fast as the *Bacillus leprae* in tissues.

Campana from 1889 onwards has described micro-organisms, which he cultivated anaerobically from the nodules of lepers. I have been able to examine the culture: it is a mixture of cocci, and coli-like organisms, none of which are acid-fast or even acid-resisting. There appears to be a sad lack of technique in isolating these cultures, an opinion also apparently shared by Wasserman.

Kanthack and Barclay, in 1891, cultivated a micro-organism of varying acid-fast properties from a leproma. Originally they considered it to be leprosy, but later on they withdrew their claim.

Levy in 1898 cultivated from a leper a slightly acid-fast, filamentary organism, which in later publications he considered identical to that isolated by Czaplewski in the same year from another case of leprosy. No animal experiments succeeded with these micro-organisms.

Spronk, also in 1898, was successful in isolating a diphtheroid from lepromas. He carried out some agglutination experiments with it, which, however, appear

not to have had conclusive results owing to incomplete knowledge of the agglutinating properties of sera at that time.

Babes in 1899 isolated on several occasions, from nodules and the inner organs of lepers, an acid-resisting diphtheroid, illustrations of which he has given, and which I consider extremely suggestive. Unfortunately he did not follow the work up, as he later considered that these diphtheroids were contaminations.

Barannikow in 1899 cultivated also a diphtheroid from lepers, very similar to the one isolated by Bordoni-Uffreduzzi, Spronk, Babes, Levy, Czaplewsky. This he considered was a pleomorphic stage of Hansen's "bacillus."

Kedrowsky published his first results in 1900. Using placental-extract-agar, he succeeded in isolating from the nodules of lepers an extremely pleomorphic micro-organism, with varying acid-resisting properties.

Weil in 1906 succeeded in getting what he thought was a multiplication of "bacilli" by injecting leper serum into a hen's egg, which he incubated at 37°C. He was not able to carry this further than one, the first, generation.

Rost published his first results in 1904. He made a great point of the fact that the least trace of salt would not allow the culture to succeed. The medium used was the extract from rotten fish. Semple in 1905 was sent to confirm Rost's results and found that his isolations were due to faulty bacteriological technique. This was also the opinion of other bacteriologists who had occasion to examine his work.

Rost's culture is a dark red, wrinkled, dry culture. It is markedly chromogenic, especially on gelatine at room temperature, under which conditions it grows quite easily. At 37°C. it grows rapidly on most of the usual culture media, sometimes within 48 hours.

Morphologically it is a slightly acid-resisting streptothrix, with acid-fast fragments; this property varies, however, in different culture tubes. It is Gram-positive.

It resembles Grassberger's isolation from butter in culture and under the microscope. It has not produced leprotic lesions in animals.

Shiga (1909) isolated on potato-serum-glycerine a pleomorphic, partially acid-resisting diphtheroid.

Clegg in 1909 published his results and concludes with the following summary:

1. The leprosy bacillus was first cultivated from leprosy material in symbiosis with other unidentified bacteria and amoeba, and later from other cases in symbiosis with amoeba and cholera vibrios.
2. By heating a symbiotic culture of amoeba, cholera, and leprosy for half an hour at 60°C. and incubating, the leprosy bacillus was obtained in pure culture.
3. The leprosy bacillus, isolated in this manner, grows readily on the ordinary laboratory culture media.
4. The bacillus is pathogenic for guinea-pigs, subcutaneous inoculations having caused lesions, which macroscopically and microscopically resemble the leprosy lesions of the human subjects.

Clegg's culture is granular, dry, yellow, grows at room temperature on gelatine or any of the common laboratory media. I have found difficulty in getting the strain to grow in the incubator. It is acid-fast, alcohol-fast, and Gram-positive. Morphologically it consists of short plump rods, very similar in appearance to the timothy grass bacillus; at times, however, it may resemble the "Smegma bacillus," and accordingly consist of slender matted rods.

On injection into animals it does not produce lesions comparable with leprosy in man.

Currie, Brinckerhoff and Hollmann in 1910 succeeded after several failures, in growing acid-fast rods in symbiosis with cholera vibrios and amoebae. They divided up their cultures into four "strains," and found that one was practically identical with Clegg's culture from Manila.

Serra (1910) cultivated anaerobically from several cases (three of seven attempted) a peculiar micro-organism which is Gram-positive, but not acid-fast, as it can be bleached by 2 per cent. nitric acid. Morphologically it appears to be a "diphtheroid."

Twort in 1910 by using his "ericoline" method isolated from the nasal discharge of a leper, on egg-medium to which he had added ground-up tubercle bacilli, an acid-fast rod, which grew very slowly indeed, and was morphologically identical with the "bacilli" found in tissues. He was not able to carry the culture further than the first generation, but still his incomplete results deserve consideration, in view of his success with John's "bacillus."

Duval in 1910 and the following years published various results in connection with the isolation of Hansen's "bacillus."

His first publication was founded on Clegg's results, and the isolation of the acid-fast micro-organisms was made with amoebae in the fashion identical with that of Clegg. The micro-organism in question appeared, however, to differ from that isolated by the latter. In later publications Duval found he could dispense with the amoebae and cultivate the "bacilli" from the nasal discharge of lepers simply by adding amino-acids to the culture media. Later on, even the addition of the amino-acids does not seem to be indispensable, for an isolation is mentioned on Novy-Mac Neal's blood-agar. The last papers deal with isolation by means of Kedrowsky's placental juice agar, which had been re-discovered by Wellman and published as "Wellman's placental agar." Animal experiments failed to produce typical leprosy lesions.

Duval's so-called culture of lepra is a bright yellow, moist, smooth culture. It grows easily and abundantly on gelatine by room temperature and can be easily regained in pure culture on common agar from the organs of animals that have been inoculated with it. At 37° C. it takes about three days to grow on any of the current laboratory media.

Morphologically it is coccoid in appearance, but under circumstances, when injected into animals and sometimes on artificial media, it is apt to present a somewhat more elongated appearance, but at no time closely resembles Hansen's "bacillus."

It is acid-fast and alcohol-fast. Gram-positive.

Its cultural and morphological characteristics cause it to resemble markedly a saprophytic acid-fast micro-organism isolated by Nabarro from London milk. Therefore it is not astonishing that animal experiments did not succeed in producing any lesions analogous to those found in lepers, and that at the International Medical Congress in London (1913) this author admitted he had no culture of leprosy to show or hand round.

Kedrowsky's paper published in 1910 deserves special mention in view of the care with which the experiments have been carried out. They took ten years to complete.

His first isolations from the nodules of three lepers gave as a result two distinct bacteria; one was a non-acid-fast filamentary, interlacing, branching micro-organism, the other was a slightly acid-resisting diphtheroid. He also cultivated

other micro-organisms, which, however, from his pictures and descriptions are easily seen to be but variations of these two groups. He injected these various bacteria strains singly, into mice and rabbits, in different fashions (under the dura, intravenously and intraperitoneally) and observed the animals for prolonged periods, in some cases over two years. He found that, whatever micro-organism he had injected, the result was practically the same in all cases; very numerous acid-fast micro-organisms of the "tubercle bacillus" type in the viscera, from which they could be regained in pure culture as acid-fast rods. The resulting lesions resembled in some cases the chronic type of tuberculosis induced in rabbits by injections of human tuberculosis; in others the lesions were strikingly similar to those occurring in visceral leprosy of human beings.

Kedrowsky's acid-fast culture of "Hansen's Bacillus" is a moist, creamy, smooth or wrinkled ivory-white culture which resembles avian tuberculosis to a very marked extent. It grows only at incubator temperature on special media, such as placental-juice-agar, glycerine-agar, horse-serum-nutrose-agar or any similar medium suitable for tubercle. Multiplication is generally apparent within ten to fourteen days, but may take three weeks to four weeks or more to attain its maximum.

No growth takes place at room temperature or on gelatine.

It is acid-fast, cannot be bleached by 20 per cent. nitric acid in one minute after staining for five minutes with warm carbol-fuchsin. It is alcohol-fast, will withstand absolute alcohol for ten minutes. Gram-positive. Morphologically it is markedly pleomorphic, and resembles avian tuberculosis in this respect, in fact I do not believe that these two bacteria can be distinguished from one another under the microscope, except that Kedrowsky's strain of lepra is more apt to show clubbed shapes than even avian tubercle.

Williams in 1911 isolated diphtheroids and streptothrices from the nodules of lepers. He considered them to be pleomorphic stages of Hansen's "bacillus," but was not able to produce any typical lesions in animals with the micro-organisms isolated.

Currie, Clegg and Hollmann express their opinion on these cultures in the following terms:—

"This investigator considers that his work tends to confirm the work of Rost, Deycke, and Clegg, i.e., that all these investigators have, by different methods, isolated the same organism, which latter is very pleomorphic. He seems, however, to base this opinion on very little data other than that the organisms in question were all grown from lepromata, and that local nodules were produced in animals by inoculation of some of these cultures."

Bayon (1911) published the first confirmatory results of Kedrowsky's isolations, above all in relation to the existence of filamentary non-acid-fast micro-organisms in cultures taken with all aseptic precautions from lepromatous nodules. He also confirmed the possibility of isolating on various media diphtheroids which were slightly acid resisting. Both bacteria acquired acid-fast properties after injection into animals. This author considered that the identification of bacteria isolated from lepers was the most important point in connection with the bacteriological work, and accordingly carried out extensive investigations by means of numerous experiments on animals, serological tests and observations on human beings. He found that Kedrowsky's view that the parent form of Hansen's "bacillus" is a filamentary, interlacing, branching, non-acid-fast micro-organism is correct, and that this pleomorphic property is shared by several other acid-fast bacteria, e.g., the smegma "bacillus," some saprophytes, such as the acid-fast rod found in tap-water, etc. His comparative experiments with Duval's, Rost's, Clegg's cultures

persuaded him that they behave in culture, on common gelatine at room temperature, and in animal experiments, like the usual ubiquitous, acid-fast micro-organisms found in grass, dung, milk, butter, earth, etc. This observation he confirmed by serological tests, which showed that these cultures when used as antigens do not react specifically with any lepers' serum. The lesions produced in animals are not like the lesions of rat or human leprosy. On the other hand, he found that, given sufficient time for incubation, and notwithstanding a very heavy percentage of failures, Kedrowsky's strain was capable of producing in rats, mice and rabbits lesions extremely similar to those occurring in visceral lepra of human beings. To obviate as much as possible any experimental error, the culture was injected into guinea-pigs and fowls to determine whether, as Babes had suggested, it might not be a tubercle strain which had infected Kedrowsky's animals. Small doses (five to ten loopfuls) did not cause any lesions in guinea-pigs or fowls, even after prolonged observation.

The serological tests employed also showed this strain to be capable of reacting specifically with the serum of certain lepers. A filtered cultural extract when injected into lepers caused a rise of temperature to take place. This reaction, however, has no specific value, as it can be brought about by many different substances, and is, moreover, not constant.

Bayon's culture (in the diphtheroid stage) was studied by Priestley, who compared it with numerous (namely, 48) other micro-organisms from various sources, showing somewhat similar morphological features, with the intention of bringing evidence to bear on the point, whether this diphtheroid could possibly be an ubiquitous saprophyte, whose presence in the skin of lepers had only casuistic, and not etiological importance.

Priestley found that morphologically the "bacilli" were very irregular in shape, clubbing was very marked, polar granules could be detected by Neisser's stain. Gram-positive. On serum they formed raised, white circular colonies, like Klebs-Loeffler "bacillus"; on agar, very minute, circular, greyish-white colonies; no growth on gelatine; on broth, very slight growth, very stringy deposit; no growth on potato. Non-virulent. They fermented dextrose and laevulose, but not saccharose, lactose, maltose, mannite, dulcitol, glycerine, dextrite, galactose, did not clot milk. Unfortunately the study was not completed at the time, and no further details were published because all attention had to be concentrated in attempting to bring the peculiar results of some American authors in relation with the observations made on Kedrowsky's culture and the acid-fast micro-organism isolated from rats inoculated with acid-labile cultures from lepers.

Numerous experiments on animals showed, however, that the acid-fast stage of the filamentary micro-organism was capable of producing in rabbits, rats and mice lesions very similar to those obtained by Kedrowsky in his experiments, and which accordingly were analogous to the lesions observed in leprosy of the human being.

The result of these numerous experiments, prolonged over several years (4), was accordingly that Kedrowsky's isolation was true leprosy, and that the cultures of some of the other authors appear to be accidental.

Reenstierna (1913) succeeded in cultivating from the blood and nodule of a leper an acid-fast rod, which was morphologically similar to Hansen's "bacillus" seen in tissues, and numerous acid-labile bacteria. The acid-fast rods were isolated in pure culture by the means of 10 per cent. anti-formin, and lasted four generations in their acid-fast condition, and then turned acid-labile.

A pure culture isolated from these acid-labile bacteria showed on single occasions the presence of acid-fast micro-organisms.

A monkey (*Macacus rhesus*) was injected in the brain and ischiatic nerve with a pure culture of the acid-fast rod isolated from the blood of a leper. This animal showed peculiar maculae on the chest and face, which contained acid-fast micro-organisms. The death of the monkey took place three months after injection, and the inner organs showed numerous caseating nodules which contained big bundles of acid-fast rods, which were mostly situated extra-cellularly.

Another *Macacus rhesus* was injected with a pure culture of the acid-labile micro-organism isolated from the blood of a leper. Forty-two days after, it developed blisters on its fingers and toes, which contained acid-labile and acid-fast bacteria. Shortly after the animal was killed, and acid-fast rods were found at the site of injection, and in caseating inguinal and sacral lymphatic glands.

A third *M. rhesus* was injected with a culture of the acid-labile micro-organism, which was isolated from a single bacterium by means of Burri's Indian ink method. This animal lived eight months, and though during its life it developed a spot which contained diphtheroids and a contracture, the autopsy revealed no leprotic lesions.

Reenstierna concludes from his numerous observations that the bacillus of leprosy is not only morphologically but also biologically closely related to the tubercle "bacillus."

His experiments deserve special consideration, because they belong to the few which have been undertaken without the preconceived *idée fixe* that leprosy and tubercle are absolutely distinct diseases in every single particular, and that Hansen's "bacillus" is acid-fast from beginning to end of its existence, notwithstanding that there is sufficient evidence to show that either statement need go unchallenged.

Stanziale (1913) cultivated from the eye of a rabbit, which had been inoculated 128 days before with a fragment of leprotic nodule under anaerobic conditions, on agar-egg-yolk, an acid-fast rod very similar to that of tuberculosis. The original culture took a month to develop, but on other media the colonies were already apparent after twenty-four to forty-eight hours. On agar and broth the culture resembles tubercle, except for the fact that it is more abundant. Apparently the resistance to the bleaching properties of acids varies, though the bacterium contains granules which are markedly acid-fast. Gram-positive. Numerous clubbed shapes similar to those seen in *Corynebacterium diphtheriae*. Non-pathogenic for animals.

Wolbach and Honeij (1914) have lately isolated a diphtheroid from a case of leprosy. It was originally cultivated aerobically on ascitic fluid dextrose agar, and appeared within ten days in the form of a translucent whitish band around the piece of gland tissue.

"When first isolated the growth was poor on bouillon, with or without glycerin, and on plain agar, dextrose agar and glycerin agar. After several months' cultivation the bacillus grew readily on these media. No growth has been obtained upon potato, with or without the addition of 0.5 to 1 per cent. sodium carbonate. Glycerin does not favour the growth of this bacillus." Gram-positive. After staining with carbol-fuchsine (how long?) they resisted decolourization with 1 per cent. hydrochloric acid in 70 per cent. alcohol; 20 per cent. sulphuric acid left the bacilli from litmus milk distinctly red, after staining by Gabbett's method for tubercle. No lesions were obtained on inoculation into Japanese waltzing mice, guinea-pigs, rabbits and white rats. The methods of inoculation were intravenous, subcutaneous and intraperitoneal.

Wolbach and Honeij consider this culture to be identical with the diphtheroid which has so often been isolated from cases of leprosy, and put in a plea for more full data about any micro-organisms which may be cultivated in future.



These are the results obtained in the course of upwards of thirty years of bacteriological investigation of an extremely widely spread disease. No doubt hundreds of negative results have been obtained and not published, but it can be said that as a rule every time the problem has been tackled by a competent bacteriologist the culture and isolation of a diphtheroid or filamentary germ has been the result. This has also apparently been the case with the investigations of Fraser and Fletcher, who, however, discarded all diphtheroids because of their ubiquity, a standpoint which would considerably simplify pathology. The micro-organisms of syphilis, of tuberculosis, of cholera, could also, on similar grounds, simply be ruled out of court, as far as their etiological significance is concerned.

The main point has, however, always been that as a rule these diphtheroids, though slightly or partially acid-resisting, did not turn absolutely acid-fast on injection into animals, with the exception of the instances mentioned.

It should be understood that every experiment connected with leprosy should be repeated scores of times, because evidently if the matter were simple it would have been solved from the very beginning. The crucial experiment to be carried out with any culture isolated from a leper is not only to get it to acquire the acid-fast properties and the morphology of the 'bacillus' seen in tissues, but also to succeed in producing lesions analogous to those seen in lepers and in rats spontaneously infected with *M. leprae muris*. Bearing in mind the great difficulties encountered in transmitting leprosy to animals through the injection or inoculation of nodules teeming with 'bacilli,' we must be prepared to face series and series of negative results. As negative results, however numerous, are powerless to destroy a single positive observation, it must be admitted that Kedrowsky has published micro-photographs and drawings of lesions in rabbits and mice which are strikingly like those produced by the inoculation of human and rat 'virus' in rabbits and rats. Such specimens have not been published by any other author, excepting those to be found at the end of this paper; the corresponding deduction should not be difficult. (Plate II, fig. 11.)

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## VI. THE EXPERIMENTAL TRANSMISSION OF LEPROSY TO ANIMALS

Leprosy is definitely known to be a bacterial disease, and therefore it should not and cannot be impossible to transmit it to animals by experimental inoculation, if it is considered that practically all pathogenic bacteria can be made to produce in animals diseases similar or analogous to those caused in man. In fact, the existence of a disease in rats, extremely similar to leprosy, ought to throw light on this vexed question and postulate the possibility of the experimental infection of laboratory animals.

In estimating the value of any experiments made for the purpose of inoculating animals with leprosy, it should be remembered that but fourteen years ago it was universally denied that syphilis could be given to rabbits or monkeys. Even scientists like A. Neisser upheld this belief, which, as we now know, was absolutely erroneous and totally unfounded. Syphilis is extremely contagious, develops comparatively rapidly, therefore it is not astonishing that

the transmission to animals should be a relatively easy matter, much more so than leprosy. Yet, even after the communication of the first successful inoculations of syphilis to rabbits and monkeys, numerous negative experiments were published, accompanied by the statement that the sores produced on the testis of rabbits, etc., were not produced by the syphilitic virus but were due to the effects of contaminating bacteria.

These recent happenings should induce more attention being paid to the evidence which has accumulated in the course of time, showing that though in the great majority of cases leprotic products, when inoculated into animals, are eliminated without leaving any definite traces, still multiplication and conclusive dissemination of the bacilli does take place in a small number of instances.

In 1881, Neisser, after twenty-four unsuccessful attempts on rabbits, injected two dogs with leprous nodules, and concluded from the results that leprosy developed locally at the site of inoculation.

1883. Damsch grafted lepromata in the anterior eye chamber of two rabbits. The animals died after 139 and 219 days. Apparently a slight increase in the quantity of acid-fast germs had taken place. He also inoculated two cats in the abdomen with leprous nodules. After 120 days numerous bacilli were found in the newly-formed tissue round the atrophied nodules.

1888. Vossius carried out experiments in a similar fashion to Damsch on rabbits and had identical results. He also considered that the acid-fast germs had multiplied.

1885-6. Melcher and Ortmann inoculated four rabbits in the same way. They succeeded in getting, after four to ten months, definite metastases and deposits of acid-fast germs in the spleen, liver, caecum, pleura, and pericardium. They stained these germs with Baumgarten's differential stain, and found that they had the staining properties of Hansen's "bacillus."

1887. Wesener injected several rabbits with leprous nodules, and in two animals out of eight, after periods of four and a half months and eight months respectively, he found lesions apparently identical with those described by Melcher and Ortmann—that is, nodules from the size of a pinhead to that of a pea in the lungs, on the pleura, on the epiploon, in the liver, in the lymphatic glands, spleen, kidneys, caecum, and peritoneal surface. Caseation and giant cells were present. In the eyes of the rabbits he obtained after six and eight months a congregation of round cells choked up with acid-fast micro-organisms. He considered that the generalised lesions were due to tuberculosis, and that the ocular lesions were caused by dead bacilli, because acid-fast germs can be demonstrated in the eye of the rabbit after inoculation with nodules which have been kept for years in alcohol.

1901. Barannikow confirmed these results on one rabbit.

1893. Wnoukow inoculated twenty rabbits with leprous nodules in various fashions (intraocularly, subcutaneously, intraperitoneally). In fourteen rabbits he got lesions which he considered to be of tuberculous nature. No confirmatory tests with guinea-pigs appear to have been made.

1893. Tedeschi inoculated a leproma into the dura of a monkey. The animal died after eight days; that is far too brief a period for any conclusions to be drawn.

1902. Ivanow injected several guinea-pigs with leprous nodules. In one case the animal was killed after eight months and found to have nodules in the epiploon, which Ivanow considered were due to multiplication of the bacilli.

1905. Thiroux inoculated five rabbits with leprous material. The animals lived thirteen to twenty months, and presented various lesions at autopsy, which Thiroux considered to be tuberculous, after positive inoculation of two guinea-pigs.

1906. Nicolle inoculated monkeys with ground-up nodules, and succeeded in getting localised lesions.

1909. Marchoux and Bourret inoculated a chimpanzee under the skin of the ear with a freshly-excised leprous nodule. It apparently increased in size during three months, and then began to be resorbed. An examination of the blood showed a few leucocytes with badly-staining bacilli. The animal died ninety-six days after inoculation. The nodule had reached the size of a split pea, and under the microscope was shown to consist of three layers. In the middle were the remains of the original tissue, quite necrosed, with a few loose acid-fast germs. Around this was a considerable number of lymphocytes, enclosing numerous bacilli, and at the periphery organised connective tissue with a few cells filled with bacilli. The authors considered that a proliferation of the original germs had taken place.

1909. Sugai inoculated Japanese dancing mice intraperitoneally with an emulsion of fresh leprous nodules, and found as a result the development of miliary granulomata on the peritoneal lining, especially in the hepatic region and epiploon. Also the bronchial and peritoneal glands showed characteristic leprous lesions. All contained acid-fast germs. The attempt to transmit the disease from these to other mice failed.

1909. Kitasato appears to have successfully inoculated an orang-outang on the cornea. Only scanty details were, however, given.

1909. Stanziale reports upon experiments on rabbits. In one typical case he inoculated into the eye of the animal a fragment of leproma. During the first few days a marked hyperaemia developed round the opening in the cornea. Soon afterwards a constant diminution in the size of the nodule could be made out, accompanied by the formation of an opalescent exudation, which took its departure from the inoculated piece of tissue and spread out as a fan-shaped segment. After twenty days the nodule became stationary, and remained so during fifteen to twenty days without any noticeable change taking place. After forty days the nodule began slowly to increase till it had trebled its original size. In the same time small grey nodules made their appearance, and could clearly be distinguished on the surface of the inoculated tissue. These little nodules attained the size of a pin's head. Some appeared to be adherent, while others were loose and independent. After the initial hyperaemia due to the operation had disappeared, a new formation of blood vessels took place, which spun a delicate network round the piece of tissue inoculated. After seventy days the rabbit's eye was enucleated, part of the tissue was used for histological observations and the rest was grafted into the cornea of two other rabbits. In one the graft got resorbed without any further results in the course of two months. In the other rabbit the graft began to increase in size after fifty days, and produced similar changes to those described in the foregoing animal. The experiments are being continued by the author, but to date the results obtained are: thirty-one rabbits have been inoculated, and in eight it was possible to produce leprous ocular lesions. In some of the positive cases the

material was taken eighteen to nineteen hours after excision from the patient. In all cases in which the inoculation succeeded the Wassermann reaction was positive. As a control Stanziale inoculated lepra nodules into the abdomen of another rabbit, and found at intervals after a month the reaction constantly negative. The same negative result was achieved after grafting into the cornea of rabbits pieces of human skin or dead leprous nodules.

These experiments of Stanziale have been most carefully carried out, and full descriptive details are given; the only defect is, however, that no metastases in the inner organs are mentioned.

1911. Duval inoculated a series of animals—namely, four rats, four white mice, and four Japanese dancing mice—with grumous material taken from an acute case of human leprosy which had developed numerous soft subcutaneous leprous masses and had repeated attacks of leprous fever. A small quantity of material was used in each case—namely 0.5 c.cm. emulsified in 1 c.cm. of normal saline solution. Some were injected intraperitoneally, others subcutaneously.

The two white mice which received intraperitoneal injections died fourteen days after the inoculation. At the autopsy both showed a general infection of the peritoneum with a pure growth of the *M. lepræ*, while the mesentery, omentum, visceral and parietal peritoneum contained numerous minute, firm greyish-white nodules, which on microscopic section proved to be typical leprous lesions. The most surprising feature was the occurrence of a slightly turbid, semigelatinous exudate, which microscopically consisted almost entirely of large mononucleated cells (macrophages). Great numbers of these cells were filled with acid-fast bacilli, and scarcely any were found that did not contain a few. No remarks are made about the other animals.

1911. Couret fed two goldfish on teased nodules from a leper. No other food was allowed the animals until they had disposed of the leprosy tissue, after which they were cleansed and transferred to a clean aquarium.

The first was examined twenty-four days later; acid-fast organisms had not been found in the faeces for three days before the animal was killed. No evidence of infection was apparent externally or internally. Films made from the omentum and other organs showed a few scattered acid-fast bacilli. The average number of bacilli to the slide was about four, and no change in morphology was seen. The second fish was examined after thirty-seven days. The findings were similar to those in the first one. With reference to these experiments, it should not be forgotten that tap water appears to harbour acid-fast germs frequently, as shown by Brehm, Beitzke, Schern, and Dold. The tap water at the Lister Institute in London and of the Public Health Laboratory in Cape Town, also contains acid-fast germs; I have also seen them in the taps at Stockholm. (See Plate IV, fig. 20.)

Serra (1911 and 1913) describes a series of experiments he has carried out on rabbits by means of the intra-ocular method.

His results on the whole seem to correspond to those of Stanziale, but it is extremely difficult to unravel any definite statements as to the facts observed from a mass of secondary detail. Too much stress seems to be laid on the Wassermann reaction observed in some of the rabbits inoculated, and no details are given as to way in which it has been carried out; whilst also in this case no metastases in the inner organs are mentioned.

It is to be hoped that other following publications will bring more light on the subject.

Ch. Nicolle and Blaizot succeeded in producing in *Macacus sinicus*, by inoculating fragments of leprous tissue, local lesions very similar to those of dermal

leprosy in man. They repeated these experiments on three monkeys (bonnet chinois) and one chimpanzee, and reported on the results in 1911.

One monkey received sixteen inoculations *in toto*, but none of them ever generalised, and the resulting nodules got slowly resorbed in the course of time.

A second monkey died without showing any visceral lesions. The experimental lepromas were in course of development, and showed numerous acid-fast micro-organisms under the microscope.

A third monkey received nine inoculations at intervals varying between a week and twenty-five days. The resulting nodules softened, caseated and broke down. The pus showed a few degenerated "bacilli" among numerous polynuclear cells.

The chimpanzee received eight inoculations in the course of four months. One injection was followed by the development of a local nodule, which was followed later on by several little nodosities, which soon became confluent. This lesion only lasted about a fortnight and then got resorbed.

In these cases, therefore, we had a temporary or negative result in most cases, and a localised success in one experiment.

Bayon (1912) injected four rats on two occasions in the testes with ground-up nodules from a case of leprosy from Mauritius. Two rats did not show any macroscopic lesions, even after four months. One rat, however, developed, four weeks afterwards, a nodule at the site of inoculation, which grew to the size of a small pea. On puncture it showed acid-fast germs and necrosed tissue, some of which, no doubt, represented the original cells injected. After five weeks the rat died, but no acid-fast micro-organisms were found in the organs. Microscopical sections were made of liver, spleen, and testes. The disease, if transmitted, was localised. The whole of the nodule was then injected subcutaneously into two other young rats. Three months after inoculation, one of the rats was losing fur on the surface of its abdomen, without there being any signs of the usual rat scabies about the ears or tail. Small shotty nodules could be felt under the skin.

The other rat developed, three months after the second injection, a nodule in the left testis. This broke down, and was found to contain acid-fast rods in great quantity. This rat was killed four months after inoculation, the slight wound caused by the nodule breaking down having healed. At the site of injection and in the corresponding inguinal glands numerous acid-fast rods were found. One of these small glands was inoculated into the testes of another rat, and on killing it six months afterwards no acid-fast micro-organisms were found in the testes, but definite deposits were present in the inguinal glands, spleen and liver. A multiplication must have taken place, because the acid-fast bacteria detected in these organs were evidently more numerous than the relatively small quantity injected. (Plate III, Fig. 14.)

At Robben Island, during 1912, 1913 and 1914, thirty experiments on rabbits were undertaken by means of the intra-ocular method. Exact data will be published in due course, when the histological study of the resulting specimens is undertaken.

It is, however, worthy of note that one single rabbit of the thirty inoculated has shown permanent lesions—that is, minute white, vascularised nodules in the cornea and on the iris. This animal was operated upon 1st November, 1912; nodule from Pat. No. 1294.

As a rule the remaining animals developed inflammatory appearances similar to those described by Stanziale, but in the course of time the inoculated fragment of leprotic tissue got resorbed, all signs of inflammation or production of new tissue subsided, and in conclusion only a linear scar remained as a trace of the operation.

One animal, however, still shows numerous minute leprotic lesions one year and seven months after inoculation.

I do not wish to admit a definite communication of leprosy to these animals, till I have been able to achieve the results accomplished with the rats—that is, the transmission of the virus through several generations, and deposits in the inner organs.

Reenstierna (1912) injected a *Macacus rhesus* under the mucous membrane of the nose with 0.5 c.cm. of an emulsion made from a leproma containing numerous “bacilli.” The animal died forty-two days after inoculation. The inner organs showed no alteration, but the nose had developed a perforation of the septum; moreover, there was extensive ulceration of the upper lip and nasal mucous membrane. The microscope revealed numerous extra-cellularly situated acid-fast rods in the nasal secretion and sections of the diseased tissues. In addition an acid-labile but Gram-positive filamentary germ was present with fragments which, morphologically, were similar to lepra “bacilli.”

Another *M. rhesus* was injected intra-peritoneally and subcutaneously with the same emulsion as in the previous experiment. The monkey died fifty-nine days afterwards, and showed numerous caseating nodules in the liver, spleen, lung and mediastinal lymph-glands. These lesions contained numerous acid-fast rods.

Five guinea-pigs were inoculated with the caseating nodules from the spleen; they died within three to five weeks and showed localised lesions which contained very numerous acid-fast rods.

A third *M. rhesus* monkey was injected with the same emulsion intraperitoneally and subcutaneously. It died six months afterwards, and developed small nodules at the sites of injection which contained acid-fast rods. A cherry-sized nodule which developed on the upper-lip contained numerous acid-fast intra-cellular “bacilli” and vacuolated cells. Acid-fast were also found in the liver, spleen and bone-marrow.

These are the successful or reputed successful attempts to transmit leprosy to animals by means of injection or inoculation of leprosy tissues.

As will be seen, they are certainly more numerous and have been more carefully observed, than the isolated statements in connection with the communication of syphilis to animals, which were to be found in literature only so short a time ago as the first year of this century. And yet syphilis can be communicated to rabbits with relative ease. In dealing with leprosy, where even direct inoculation of human beings has failed (except in Arning's case) one can but expect to find much more serious difficulties, such as:

(1) Lack of knowledge in relation to the bionomics of Hansen's ‘bacillus.’

It does not seem to be understood that though attendants in leper asylums, and persons living with lepers, must often come in contact with numerous ‘bacilli’ which we know nodular lepers cast



about them, yet only a very small proportion ever develop the disease in a clinically recognisable form. In other words, in the great majority of cases either the 'bacilli' remain absolutely quiescent in the body or get harmlessly eliminated.

Therefore it should not astonish us that a great proportion of the animals inoculated eliminate the acid-fast bacteria injected after a longer or shorter period.

(2) The difficulty of proving whether any 'bacilli' seen in experimental lesions are simple deposits of dead acid-fast bacteria or whether they are alive and in course of multiplication.

This is really an outcome of No. 1, as it depends on an incapacity in comprehending how extremely passive, inert and resistant Hansen's 'bacillus' is.

It is true that Campana, Wesener and others have shown that it is possible to bring about lesions in animals very similar to those described by the experimenters who claim having transmitted leprosy to rabbits or rats, by simply injecting tissues containing numerous 'bacilli' which had been autoclaved or kept in alcohol for months. As we know of no method of cultivating directly the acid-fast germs seen in leprosy with their original morphology and staining properties, it is of course difficult to refute this argument except by pointing to the analogy with rat-leprosy, where, though we know that the 'virus' used in experiments is living because it can be indefinitely transmitted, still, except for the latter particular, the lesions caused by killed and living virus are extremely similar.

(3) The similarity existing between the lesions produced in rabbits and those found in experimental tuberculosis.

This is the argument which settled the results of Melcher and Ortmann, Wesener and others. At the time leprosy and tuberculosis were considered to be as easily distinguishable histologically and bacterioscopically as they are clinically. Further study has, however, shown that this is not the case, and that under certain conditions only experiments with guinea-pigs can decide whether we are dealing with lesions brought about by Koch's or Hansen's 'bacillus.'

Taking into careful consideration the previous experiments and personal experience, in addition to the interpretation of the clinical notes made on lepers who have been under observation for fifteen

and more years in South Africa,\* no other conclusion can be arrived at than that leprosy is directly, experimentally, communicable from human beings to rats and rabbits; that a long time of incubation must be allowed for; that the great majority of animals eliminate the 'bacilli' injected after a shorter or longer period of quiescent deposit.

We have, therefore, under experimental conditions an analogous counterpart of what is happening daily with people who contract leprosy, for even prolonged exposure to infection causes the disease to develop only in a small percentage. Those who show clinical symptoms of the contagion appear to eliminate the majority or practically all their 'bacilli' if they live long enough to become what is called an 'arrested' case. Such an 'arrested' case is a leper who is known to have had numerous visible lesions teeming with acid-fast rods, but who for the time being only shows scars and traces of the disease with no 'bacilli.' Such cases relapse at times, or may show small bacillary deposits in their inner organs at death, but even then there can be no doubt that they have succeeded in eliminating or destroying millions and millions of 'bacilli' from or in their nodules.

A somewhat analogous observation is often made at autopsies in connection with tuberculosis of the lungs. It is then seen that very many bacillary infections of this organ do not develop into clinical tubercles, but heal with scarring.

All these considerations should, and do, give a satisfactory explanation of many negative results and condone the scepticism with which many successful experiments have been received. Conclusive histological specimens like those shown in Plate III, fig. 14 cannot be ignored, especially as experimental results agree in an exceptional manner with clinical observation.

Once more, these conclusions have to be arrived at:

(1) Leprosy is experimentally transmissible to animals such as the rabbit and the rat. Allowance must be made for the fact that the great majority of inoculations do not succeed, owing to the 'bacilli' being eliminated or destroyed without leaving any visible trace.

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\* This shows that in single instances lepers get rid of nearly all the acid-fast germs from their lesions.

(2) The incubation period is very long, and the resulting lesions in the inner organs may not be visible to the naked eye.

(3) In rabbits the bacillary deposits in the inner organs resemble tuberculosis. In such cases the question can only be decided by means of guinea-pig inoculations.

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## ON RAT LEPROSY AND ITS RELATION TO THE HUMAN DISEASE

A disease extremely similar to leprosy, not only in its clinical features but also from an histological and bacteriological point of view, was discovered in rats by Stephansky in Odessa, in 1903. Its presence among the sewer rats of Berlin was confirmed by Lydia Rabinowitch the same year. Both these observers considered that rat leprosy could not be transmitted experimentally from one animal to the other, and that the acid-fast micro-organism found in enormous quantities in the lesions of the animals affected could not be cultivated on nutrient media.

George Dean had studied this same disease at Elstree, near London, for some time, and published his results nearly contemporaneously with Stephansky and Rabinowitch. He was, however, successful in transmitting rat leprosy through several generations of rodents, a result which has since been amply confirmed by Marchoux and others. G. Dean's cultural attempts only succeeded in isolating a diphtheroid, which showed filamentary forms, and much to his astonishment reacted specifically with the serum of lepers. The results of his agglutination experiments were:

- ' 1. The serum of rats inoculated with rat-leprosy, i.e., with acid-fast "bacilli," agglutinated the diphtheroid. Normal rat's serum had no agglutinating properties for this micro-organism.
2. The serum from a case of human leprosy agglutinated the acid-fast micro-organism from the rat. Normal human serum had no agglutinating power.
3. Normal human serum and the serum from a tuberculous patient failed to agglutinate the diphtheroid, whereas the serum from a case of leprosy had distinct agglutinating properties.'

These carefully planned tests, in addition to the histological appearances, the cultural and experimental behaviour of the micro-organism causing the disease in rats, lead us to believe that rat leprosy is possibly related to human leprosy in a manner similar to that existing between bovine and human tuberculosis.

Marchoux has carried out very carefully-planned experiments with the strain of rat leprosy he discovered in Paris, and has come to the following conclusions:—

- ' 1. That only six in a thousand sewer rats in Paris present marked leprotic lesions, but that 5 per cent. harbour acid-fast micro-organisms (*bacille de Stephansky*).
2. That the inguinal glands appear to be the first attacked by the disease.

3. That the lung acts as a filter in arresting the germs and then directing them towards the mediastinal glands.
4. That by simply placing the virus of the disease on the scarified or depilated skin, an infection can be brought about in rats even more easily than by subcutaneous injections.
5. That intact skin or even the bare skin of very young rats does not allow the penetration of the virus.
6. Rat leprosy is a disease peculiar to the rat. Mice can be infected, it is true, but with less ease than is the case with rats. Attempts to infect through the scarified or depilated skin do not succeed as often as subcutaneous injection in mice. In such cases one finds amongst the "bacilli" involution forms similar to those met with in rats injected with human leprosy.
7. *Mycobacterium leprae murium*, like Hansen's "bacillus" is a parasite of the mesodermal cells. It does not live at the expense of its host, but of the same substances which nourish the cell containing it.
8. Granular bacilli are dead.
9. A first culture is relatively easy to obtain. Further sub-plants are not obtainable.
10. The bacillus disappears rapidly in contaminated media (*en milieu impur*).
11. It does not resist desiccation.
12. It is not killed by a temperature of 60° C. during five minutes. It dies after being exposed at the same temperature for fifteen minutes.
13. Infection takes place through the skin, and accordingly superficial surface regions are generally more intensely affected.
14. Infection follows the lymphatic stream.
15. The point of inoculation is not always the most heavily infected spot.
16. Male rats can be infected by depositing some of the virus under the prepuce without causing any lesions of the mucous membranes.
17. However, the spontaneous disease does not seem to be transmitted in this fashion.
18. Insects do not carry the disease.
19. The sarcoptes of rat scabies play only an indirect rôle in the diffusion of the disease.
20. The immediate contact of a skin abrasion with a diseased surface or with freshly contaminated objects is the usual mode of contagion.
21. By introducing a large number of micro-organisms through the digestive tract a primary pulmonary infection can be produced.
22. The lymphatic glands of artificially inoculated rats are generally very small; those of spontaneously diseased rats, on the contrary, generally much enlarged.
23. Inoculation generally produces an infection of the lymphatic glands. Impure virus has to be injected to produce the muscular and cutaneous type of the disease.

I have quoted extensively these results of Marchoux and Sotél, because not only all experiments have been carefully and systematically worked out, but the majority of the observations made seem to agree so completely with what we know of the pathology of human leprosy.

Currie and Hollmann also carried out experiments with rat leprosy, and in two papers on the subject came to the following conclusions:

- ‘ 1. In some cases of artificially acquired rat leprosy the onset is with broncho-pneumonia, accompanied by a septicaemia and without any other demonstrable lesions.
2. In other cases of this disease pneumonia is a very early lesion, but we cannot positively state that it is always the first lesion.
3. That the animal may die in the pneumonic stage before other lesions present themselves, or it may develop pneumonic symptoms and recover from the same only to develop later the well-known lesions.
4. That during the stage of the disease in which the animal is very ill certain mites (*Laelaps echidninus*) were found to be very numerous on the animals’ bodies.
5. That during the stage of the disease in which septicaemia is marked, these mites’ digestive tracts contain the bacilli of rat leprosy in considerable numbers, and, therefore, these parasites may be one means of transmitting the disease. This latter probability is, of course, not proven.’

In a later paper, published in 1912, these authors add the following to the previous conclusions:

- ‘ 1. In the disease we are dealing with, whether the animal is inoculated by a laboratory method or simply allowed to develop the disease from coming into contact with infected rats, i.e., the natural mode of infection—the lesions met with are practically the same.
2. With the exception of the local lesion, occasionally produced at the site of artificial inoculation, infection of the viscera seems to usually precede the lesions of the skin.
3. Of the visceral lesions, a broncho-pneumonia is often the earliest and most constant. Infection of the spleen is also often an early event.
4. The heart blood of infected rats often contains the bacilli of rat leprosy, and no difficulty is experienced in demonstrating the presence of acid-fast bacilli in the mites contained on the bodies of these animals when the latter’s heart blood contains the organisms.
5. The fact that these mites contain the bacilli so frequently naturally leads one to suspect that they may be one of the means of transmitting the disease from rat to rat, but up to the present time we have no evidence that such is the case.

#### ATTEMPTS TO CULTIVATE THE BACILLUS OF RAT LEPROSY

As reported in our first paper, we have made many attempts to cultivate the bacillus of rat leprosy on artificial culture media, both on the media ordinarily employed in the laboratory and by the method of Clegg in symbiosis with amoeba and cholera. We have now to report that we have continued these experiments for nearly a year, and that our results have been entirely negative, not having secured a single culture of an organism which we considered to be the bacillus of rat leprosy. In one of our attempts to cultivate this organism from the ulcer on the abdomen of a leprosy rat, we succeeded in growing what appeared to be an acid-fast streptothrix. As one might expect to obtain such organism on an exposed surface of that kind, we regarded this culture as an accidental contamination, and not the bacillus of rat leprosy.’

It is hardly feasible to bring these results in correlation with anything observed in human leprosy, except for the fact that also these authors have found the disease can be transmitted by means of inoculation from one animal to another, an experiment which did not succeed in the hands of earlier observers. In a similar fashion the transmission of human leprosy to animals has failed time after time, and yet under exceptionally favourable circumstances it can be successfully achieved. The repeated attempts to cultivate the micro-organism of the disease, which only resulted in the isolation of acid-fast filamentary, branching bacteria (diphtheroids are not mentioned) which were considered to be contaminations, are also worth noting, because this observation coincides with the results of much of the work done on the bacteriology of human leprosy.

Bayon (1912) succeeded in isolating on fish-juice-agar from the spleen of a rat which had been injected with a ground-up nodule from a spontaneously diseased rodent, a moist, ivory-white, creamy growth, which multiplied in sub-cultures very slowly at 37° C. on the media generally used for the artificial cultivation of tubercle. This culture produced in rats the identical glandular and visceral lesions observed in rats injected with the virus from spontaneously diseased animals. The glands and organs infected were teeming with acid-fast micro-organisms, which caused little or no tissue reaction and a very minimal production of giant cells (Plate III, figs. 16 and 17).

The culture does not grow at room temperature or on common gelatine. It is difficult to isolate again from the inner organs of the animals into which it has been injected. Guinea-pigs, fowls and rabbits do not show any lesions if inoculated with small quantities (five to ten loops), but if a culture slope or more is introduced intra-abdominally, after a time caseating, necrosed nodules are produced on the surface of the peritoneum and the omental glands can be considerably enlarged, showing big masses of acid-fast micro-organisms. The necrosis and caseation are partially due, I believe, to the mechanical foreign-body action of the large numbers of resistant micro-organisms injected.

No growth could be obtained under anaerobic conditions.

In an oxygen atmosphere the cultures develop somewhat more

rapidly; after ten to fourteen days a thick growth is discernible, whilst under ordinary conditions, at 37° C., three or four weeks are needed.

No multiplication was obtained on gelatine at room temperature or on common agar in the incubator.

No pigment was produced on any of the following media: fish-juice-glycerine-agar, glycerine-agar, glucose-agar, glycerine-potato, rice-glycerine-milk, sterilised wedges of rabbit's liver + glycerine, Dorset's egg-medium. All these media were suitable for growing the micro-organism.

Morphologically the culture is a short rod, with a marked tendency to pleomorphism; clubbed shapes are frequently seen, and at times true branching can be detected. The length of the rods varies; in fact, the microscopical appearances are very similar to those of a culture of avian tuberculosis, which it also resembles from a cultural point of view.

It is acid-fast, i.e., cannot be bleached by one minute's treatment with 20 per cent.  $H_2SO_4$  after having been stained for five minutes with warm carbol-fuchsin. It is also alcohol-fast. After soaking for six hours in warm alcohol its staining properties are unimpaired.

It is Gram positive.

Very numerous attempts to isolate the same micro-organism from several other cases of rat leprosy failed completely. Marchoux is therefore not quite correct in saying that: 'Bayon, au contraire, l'aurait cultivé assez facilement.' (l.c. p. 23.)

Further cultural results have been published by Hollmann, Chapin, Zinsser and Carey, Marchoux and Sorel, and, of course, Wellman. None of these cultures have succeeded in producing leprotic lesions.

After Stephansky's and Rabinowitch's and Dean's observations, rat lepra was found in Australia by J. R. Bull, in New South Wales by Tidswell, in the United States by Wherry, McCoy, Walker, in Roumania by Mezinescu and Alexandrescu, in Japan by Jitoyo and Sakaki, in Paris by Marchoux, in New Caledonia by Leboeuf, in Ipswich by Petrie and Macalister (see Pl. I, fig. 4), in India by the Plague Commission, in Queensland by Priestley.

Though rat lepra seems to be spread nearly all over the world, still Brinckerhoff examined 16,000 rats in the Hawaii Islands without finding any trace of an animal affected with the disease.



In Cape Town I have had now 1,378 sewer rats examined without having succeeded in a single instance in discovering a marked case of the disease.\*

Dr. G. W. Robertson remembers, however, to have noticed one animal the lesions of which contained numerous acid-fast rods, in the course of plague investigations, when about 30,000 rats were examined.

Mezinescu succeeded in preparing from the ground-up nodules of leprosy rats an antigen which was capable of absorbing complement when linked up with the serum of lepers; a further proof of the relationship of both diseases, though, on the other hand, it must be admitted that the serum of lepers is similar to that of syphilitics in this respect, because it is capable of reacting with specific and certain non-specific antigens. A distinction can only be made by carefully titrating all the elements of the haemolytic system, and varying the proportions of antigen and antibody in a suitable fashion; up to the present this has only been done in quite a minimal proportion of cases.

Rats are not the only animals affected by a disease akin to leprosy. Acid-fast micro-organisms which belong to the tubercle group, but are not pathogenic for guinea-pigs in small quantities, and are only cultivable with extreme difficulty, cause diseases in cattle, horses, sheep, and possibly goats. The cattle disease has been named by K. F. Meyer 'Enteritis hypertrophica bovis specifica.'

Twort and Ingram, Vukovic, M'Fadyean, McGowan, have described 'Johne's disease' in the sheep. This disease differs from rat-leprosy in so far that the symptoms are mostly confined to the digestive tract.

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# **VII. THE SEROLOGY OF LEPROSY AS A MEANS OF DIAGNOSIS OF THE DISEASE AND FOR THE PURPOSE OF CONTROLLING SPECIFIC TREATMENT**

Soon after the adaptation of Bordet-Gengou's complement fixation to the diagnosis of syphilis by Wassermann and his co-workers, Eitner modified this test to suit the special conditions of leprosy, and prepared his antigen by means of a saline extract from a leproma. He found that the blood of lepers contained, as he thought at the time, specific anti-bodies, and accordingly was able to bring about the absorption of complement when linked up with an antigen prepared according to his method. In the course of time it became evident that Wassermann's test for syphilis could not claim to be absolutely specific. Accordingly Eitner amplified his experiments, and found that the serum of lepers was also capable of giving a positive reaction when linked up with the alcoholic extract from a guinea-pig's heart, and that therefore, also in this particular instance, the serum of lepers and that of syphilitics behaved in a similar fashion.

Numerous and various experiments were then carried out with different antigens by several investigators, and the serum of lepers was carefully compared with that of syphilitics and tubercular patients. As far as the original Wassermann test of syphilis is concerned, its relation to leprosy can be aptly summed up in the conclusions of Howard Fox (l.c.):—

- '1. A positive Wassermann reaction is frequently obtained in cases of leprosy giving no history or symptom whatever of syphilis.
2. The reaction is at times very strong, inhibition of haemolysis being complete.
3. The reaction occurs chiefly in the tubercular and mixed forms of the disease, especially in advanced and active cases.
4. In the cases of the maculo-anaesthetic and purely trophic type the reaction is generally negative.
5. The value of the test is not affected in the slightest by the results found in leprosy.'

On the other hand, if we consider the test being made with an antigen prepared in various fashions from the nodules of lepers (Eitner's test or its modifications), one finds on reviewing the literature on the subject that, with a few differences, the results mainly agree with Jeanselme's conclusions, which are the following :

- '1. Eitner's reaction applied to syphilitic sera is positive in 85 per cent. of all cases examined.
2. Eitner's reaction with the serum of healthy individuals or that of syphilitics in whom the disease is quiescent: 100 per cent. negative.
3. Wassermann's reaction applied to lepers: Practically the same conclusions as Howard Fox.'

Eliasberg has shown that the serum of lepers is at times capable of inhibiting haemolysis by itself, even small quantities as 0.4 c.c. being sufficient. A similar observation has been made by Ehlers and Bourret.

Noguchi's 'Luetin' reaction was found to be negative in eleven lepers who were free from obvious signs of syphilis (but gave a Wassermann reaction) by Clegg.

Incidentally it can here be mentioned that, according to Rocamora's and also Jeanselme's experience, salvarsan has no influence whatever on the Wassermann reaction in lepers.

Evidently neither Wassermann's nor Eitner's test can be considered strictly specific for syphilis or leprosy. It remains, however, to be seen whether a test could be devised in which by using pure cultures of 'Hansen's bacillus' a clear, specific, definite reaction might be obtained.

Currie and Clegg approached the problem with the intention of proving the identity of the acid-fast bacteria they had cultivated from lepers, and after numerous experiments concluded that:

- '1. They were unable to differentiate by the method of Bordet and Gengou the leprosy bacillus from certain other acid-fast micro-organisms.
2. Extracts of certain acid-fast bacilli, other than *B. leprae*, will deflect the complement when combined with leper's serum.
3. They were able to produce specific agglutinins for *B. leprae* by injecting a horse with the cultivated lepra bacilli.'

The explanation of these results is given by Much and his co-workers, Hössli, Lescke and Deilmann, who have shown that all acid-fast micro-organisms are more or less closely related and their chemical constituents are similar. Much sums up his results in the following words (l.c. 1912):

'We were able to show—chiefly by the reaction of immune bodies—a relationship between tubercle and lepra bacilli and other acid-fast bacilli, which is due to inherent qualities common to all these organisms. Thus tuberculous and tubercle immunised persons responded to the reaction of complement-fixation, not only with tubercle, but also with other acid-fast bacilli, though not equally with all,

and with certain sorts more than with others. A table which I have prepared shows the quantitative differences in these reactions, and according to this table the lepra bacilli have the closest resemblance to the tubercle bacilli.

In the same way leprous sera react not only against lepra bacilli, which we obtained from leprous tubercles, but they give also a specific reaction in the same scale of relationship with tubercle and (other) non-pathogenic acid-fast bacilli. These reactions are absolutely specific.'

Much's observations and experiments show clearly that, as was to be expected, the specific result of any serological test carried out with acid-fast micro-organisms is apt to be masked by the interference of a group-reaction. This is similar in its effects to that observed on trying to differentiate from a serological standpoint various bacteria of the typhoid or para-typhoid group. It is no doubt also analogous to the results of precipitation tests when being adapted to identify the blood of closely-related animals.

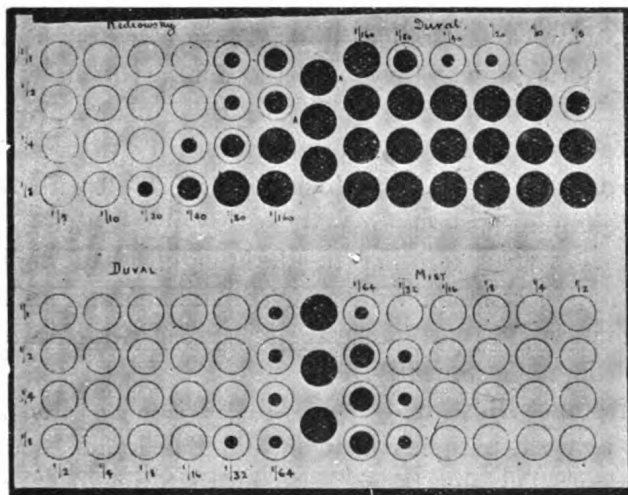


FIG. 1.

Diagram showing two complement fixation tests for the identification of acid-fast micro-organisms isolated from lepers.

Both serum and antigen are diluted in falling dosis and mixed in varying proportions.

Clear circles indicate complete haemolysis, dark circles absolute inhibition; spots of varying sizes correspond to the stages between haemolysis and inhibition.

Dark circles in the middle vertical line are controls of antigen, serum, complement.

H. R. Dean having shown that this difficulty can be obviated by linking up antigen and antibody in varying dilutions, Bayon carried out some experiments on the same lines with the intention of getting rid of the group-reaction without having recourse to

Weichardt's complicated technique. These tests require the greatest care, because the antibodies of leprosy sera are never very strong, and dilutions capable of reacting above 1/150 are the exception. The antigen was prepared by alternately freezing and rapidly thawing, and then shaking mechanically, various acid-fast cultures in distilled water. The principal factor consisted in the variation of the proportions of antigen and antibody, which can be best explained by referring to the figure on p. 49, in which the dark rings represent complete haemolysis and the clear rings complement-absorption. It will be seen that in all the rows the leprosy serum was diluted in falling quantities, 1/5, 1/10, 1/20, etc. In the first row the antigen was used undiluted, in the second 1/2, in the third 1/4, and so forth. By this method not only a control was achieved unobtainable by any other technique, but, moreover, very satisfactory and reliable results were arrived at. It was found possible to distinguish, from a serological point of view, cultures of various acid-fast bacteria, unless they were too similar or closely related, e.g., bovine and human tubercle. In this fashion Kedrowsky's strain of leprosy was found to react specifically with the serum of lepers in a dilution of antigen which gave no result with Duval's or Rost's micro-organisms.

This test can be applied to the diagnosis of leprosy, but unfortunately, in common with many other laboratory methods, it fails in those cases of early anaesthetic leprosy, which are the most difficult to diagnose clinically.

In the instance illustrated in fig. 1 the serum of a nodular leper was tried against antigens made with the leprosy cultures of Kedrowsky and Duval. It will be seen that though Kedrowsky's micro-organism reacted even in a dilution of 1/4 with serum diluted 1/40, Duval's bacterium ceased to give any absorption after mixing 1/2 with serum 1/5. These results have lately been confirmed by Kritschewsky and Bierger, who however did not grade their dilutions. In the second series of fig. 1 we see a test carried out with the serum of a rabbit which had been repeatedly injected intravenously with several slopes of Duval's culture. The serum of this animal was used against an antigen made with Duval's 'lepra,' and another consisting of Moeller's 'mist-bacillus' isolated from horse-dung. The result is that very little difference can be made

out, showing that Duval's so-called 'leprosy-culture' is very closely related to a harmless, ubiquitous saprophyte.

These results were confirmed in course by the fact that the etiological significance of this isolation had to be abandoned by its author.

Moellers (1913) examined the sera from thirty-two lepers, using as antigen the following products: Alttuberkulin Höchst, Perlsucht-tuberkulin Höchst, Tuberkelbazillenemulsion T.O.I., Neues Tuberkulin T.R. He sums up his results in the following fashion:

(1) The serum of mixed and nodular lepers gave a positive fixation of the complement in 95 to 100 per cent. of cases examined when tested against various tuberculins. Anaesthetic varieties of the disease reacted only in 25 per cent.

(2) In the serum of lepers the amboceptors for bacillary emulsions are more developed than those for preparations made from the cultural liquid.

(3) It is not possible to draw any conclusion as to the presence of tuberculosis in a leper who shows the presence of 'tuberculous antibodies' in his serum.

(4) The complement absorption properties of leprotic sera tested with tubercular substances are in direct proportion to the extent of mixed and nodular features in lepers. Accordingly it appears that this inherent quality of the serum depends on the greater or lesser spread of the leprotic lesions (Krankheitsherde); this is confirmed by the observation that the reaction was negative in arrested cases.

Kritschewsky and Bierger (1913) found that the serum of lepers gave a definite positive complement fixation test with an antigen made with Kedrowsky's culture. Duval's 'lepra' did not react in the same remarkable manner. A close similarity in relation to serological tests was found to exist between the leprosy 'bacillus' taken from tissues and that isolated by Kedrowsky. *M. tuberculosis* appeared also to be closely related to the latter micro-organisms.

These authors conclude that Kedrowsky's isolation is true leprosy, and that Duval's chromogenic culture is not etiologically connected with the disease.

Gaucher and Abrami, Sugai and others, have tested the agglutinating properties of lepers' sera towards Hansen's 'bacillus' separated from ground-up nodules. Such experiments are somewhat vitiated from the fact that acid-fast rods are prone to show spontaneous clumping.

Summing up these different independent observations it cannot be definitely stated that it is not possible, with due care and attention, to carry out serological tests showing the etiological relationship of any given micro-organism to leprosy. In the case of acid-fast bacteria the group-reaction must be obviated. The difficulties are superior to those encountered with other germs; they are, however, not insurmountable, as Harris and Langford are inclined to believe.

#### TUBERCULIN AND SIMILAR TESTS IN LEPROSY

Abraham, Goldschmidt, Babes and Kalindero, observed practically at the same time, in 1891, that lepers react with a rise of temperature to injections with 'Alt-tuberkulin.' Though this observation was repeatedly confirmed, the tendency was to consider that the reaction was due to a co-existent tuberculous infection. Babes, however, examined seven lepers after death, who during life had reacted to tuberculin, and found in four chronic tuberculosis of the lungs, whilst the remaining three were without any signs of infection with *M. tuberculosis*. Guinea-pig experiments were negative.

Sir Malcolm Morris and Colcott Fox have observed very violent reactions in anaesthetic lepers; in the former case bullae appeared all over the arms.

Babes, having given considerable attention to the subject, is of the opinion that some lepers react with quite a small dose, others only after repeated larger injections. A general reaction takes place after about twenty-four hours, in some instances, however, after eight to ten, or even as short a time as only two hours, and as a rule lasts longer than in the case of tuberculosis. The first can be followed by a second or even third rise of temperature. Under certain circumstances an extremely marked reaction may follow an



injection of less than one mmg., lasting several weeks and endangering the life of the patient.

Jadassohn has, however, been unable to produce any reaction with tuberculin in four cases he examined. It will be seen that the evidence is contradictory, but if we take into consideration the relative close relationship of leprosy and tuberculosis from many points of view, it cannot appear improbable that lepers should react to various tuberculin tests.

In previous communications Bayon was inclined to consider that intra-dermal test after Mantoux's method, carried out with a cultural extract made from Kedrowsky's strain of leprosy, might have a specific and diagnostic value. Further investigations have, however, shown that similar local and general reactions can be brought about by the extracts of several acid-fast micro-organisms such as *M. phlei*, Moellers 'mist-bacillus' and Duval's so-called 'lepra.' It is true that as a rule the reaction is not as marked with the latter substances, but the difference is only one of degree.

On the other hand, lepers who do not react to a tuberculin test may show very definite local reactions after intra-dermal injection with one c.cm. of a 10 per cent. dilution of the extract from Kedrowsky's culture. This may consist of a copper-coloured patch appearing at the site of injection or in more marked cases in the production of blisters and infiltration (Plate V, figs. 3 and 4).

One patient at Robben Island, under observation of Dr. W. L. Stuart, developed reddening and infiltration and a rise of temperature to 100° F. even after injection of minimal doses corresponding to one c.cm. of a 1/250 dilution of cultural extract. Other nodular lepers again did not react even after injection of 3 c.cm. Both extremes are exceptional; as a rule one or two c.cm. will be followed by a rise of temperature and an intra-dermal injection will result in varying degrees of discoloration. Pattison injected ten lepers (four nerve cases, four mixed and two nodular) with one c.cm. 1/10 dilution; all reacted either locally or generally. The local reaction consisted usually of a dark patch at the site of injection surrounded by a red zone, which might be swollen and tender.

All these tests have, it is true, confirmatory value, but at the present stage they are insufficient as a decisive help to diagnosis.

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### VIII. COMMUNICABILITY AND CONTAGIOUSNESS OF LEPROSY

Exact and repeated observations carried out during the last thirty years have definitely proved that leprosy is an infectious or communicable disease. The 'wisdom of centuries,' to quote Sambon's expression, had never doubted this fact. It is only due to superficial clinical observers of the XIXth century that hereditary and other irrelevant factors have lately been at all considered, even for a short time, in connection with the disease.

The statement is often made that nothing is known about the way in which leprosy is communicated from the diseased to the healthy; but as a matter of fact, the question has been carefully studied and the results are quite as definite, if not more so, than any obtained in many other infectious complaints, such as measles or scarlet fever.

Our knowledge of the contagiousness of leprosy is founded on the following observations:

(1) That it is a disease due to a definite specific micro-organism which has only been found in lepers, their excreta or immediate surroundings.

(2) That in Northern Germany, where leprosy had been re-introduced from Russia in modern times, it was seen to have spread very slowly and concentrically around the first imported cases and their contacts.

(3) That on the island of Oesel, the population of which had practically remained stationary during the last fifty-five years, Lohk, Talwik, and Dehio were able to show that out of sixty-three cases of leprosy only in eight could previous contact with lepers not be traced.

(4) That with one exception (Da Costa) all cases, in which contact cannot be proved, originate in countries or districts where leprosy is fairly common; but that in the relatively rare instances where leprosy has been contracted in countries in which the disease is not indigenous, such as the United Kingdom, Southern Germany, Holland, it is possible to prove the intimate contact with other lepers, who in their turn had contracted the disease abroad.

(5) In countries where leprosy is rare, such as the Valais, the Riviera, Alpes Maritimes, the disease is always found bound to definite foci or families.

(6) That the countries which have carried out a thorough and efficient system of segregation have been rewarded by a gradual and constant diminution of the disease. (Norway, Iceland, Germany, Sandwich Islands, Philippine Islands.)

(7) That where segregation has been abandoned, or loosely carried out, or not enforced at all, the scourge has usually attacked an ever increasing number of individuals. (India, Basutoland, Dutch Indies).

The grounds on which the contagiousness is denied are, I believe, the following:

(1) That medical men and attendants never contract the disease in leper-asylums.

This statement is not quite correct. Infection under these circumstances is not frequent, but still several cases can be brought forward showing that attendants and medical men do contract the disease. In South Africa the Medical Superintendent of a Leper Asylum contracted leprosy. Further instances of professional infection have been published by Ehlers, Vidal, Jeanselme, Nicolas, and others.

In one case of which I know, a missionary to lepers, trusting no doubt to the low degree of infectivity of the disease, allowed his little girl to play with leper children. She developed the malady whilst at school in Europe.

Published and unpublished cases of infection among religious persons who have attended lepers are also known to exist. (Three priests at Molokai, Father Boglioli in New Orleans, two nuns, a missionary, a lady missionary.)

On similar grounds it has been attempted to deny the infectiousness of tuberculosis, the deduction being based on statistics showing the low ratio of marital and professional infection.

(2) Because the disease cannot be transmitted to animals, and inoculation experiments on human beings have failed.

This also is not quite correct. The first part of this argument would have allowed us fifteen years ago to deny the contagiousness of syphilis. As a matter of fact, leprosy is transmissible to laboratory animals such as the rabbit or rat, as repeated experiments have shown. That this possibility is not more generally recognised is due to the fact that the lesions may be localised and fail to develop at all in a very great proportion of experiments, and often resembles tuberculosis to a certain extent.

As to the inoculations which Danielssen, Profeta, Cagnina, Bargilli, carried out on themselves and others, the results, it is true, were negative, but in view of the knowledge we have lately acquired of the bionomics of the leprosy micro-organism, they have only proved that experimental inoculation with small quantities of leprosy material is incapable of producing the disease in the human being in every case.

In Arning's well-known case of the convict Keanu who was pardoned on condition he allowed himself to be inoculated with leprosy, the disease did develop, but the experiment is somewhat spoiled by the fact that the man had lepers among his relatives and was in contact with them before and after inoculation.

(3) That in Norway leprosy diminished considerably in the course of sixty years, but that only about a quarter of the lepers were ever segregated at one time.

This is not quite correct; it is true that only a small proportion

of all the lepers known to exist were placed under strict segregation, that is, in special asylums, but these were the cases unable, incapable or unwilling to carry out segregation at home. All the rest were avoiding at home all contact with their relations, etc., under medical supervision. In this sense the system was one of efficient universal segregation.

A further similar argument, often brought forward by non-contagionists, is that leprosy disappeared in the Middle Ages from England, France, Germany, etc., without strict measures of segregation having been resorted to.

It appears to me that the measures adopted to prevent the spread of the disease were extremely severe. Lepers were not allowed into churches or market places, had to carry a distinctive dress, and had to make their presence known by a bell or clapper. In a few words the intimate contact between the healthy and the diseased, which gives rise to the most favourable conditions for transmission, was most radically avoided. I also believe that plague directly and indirectly swept away a great proportion of the leper population, as they were feeble, and during times of epidemics and commotion would receive no alms. This observation has been confirmed to a certain extent in India in modern times.

It will be seen that non-contagionist arguments are founded mostly on negative evidence. Positive figures bearing on the contagiousness of leprosy are available from Norway, Japan, and the Sandwich Islands, and they unanimously point to the same moral.

Kitasato's statistics from Japan show that children of lepers become leprous in a proportion of only 7.95 per cent. of the total. Matrimonial infection was proven in 3.8 per cent. cases, whilst persons living under the same roof contracted leprosy in a proportion of only 2.7 per cent. Brothers and sisters infect each other in a ratio of 4.2 per cent. These figures may need correcting according to the latest statistical methods. However, they roughly correspond to the experience gained in Norway where Sand and Lies' figures differ somewhat, but show that the children of a leprous mother are more frequently infected than those whose father alone is diseased. This proves that the more intimate contact between mother and child leads to a greater percentage of acquisition of the scourge.

Sand's statistics from Norway show that in 357 married couples in which the father alone was a leper, 1,241 children were born, of which 63, equal to a proportion of 4.9 per cent., became lepers. In 138 other married couples observed, the mother only was a leper; of 533 children born of these unions 56, or 10.5 per cent., developed leprosy. In 17 couples both parents were diseased; of the resulting 63 children 8, or 12.7 per cent., became infected.

Lies' figures from South-Western Norway give somewhat similar results. 230 married couples in which the father only was a leper had 769 children, of which 79, or 10.2 per cent., contracted the disease. In 223 married couples the mother only was leprosy; of 648 children, 106 of these, otherwise 16.36 per cent., became lepers. In 28 instances both parents were lepers; out of 79 children 29 fell victims to leprosy, that is, 36.71 per cent.

McCoy and Goodhues' observations have been made in the Sandwich Islands, and relate mostly to cases of infection noted among the kokuas or voluntary helpers of the lepers at the Molokai settlement. Their conclusions show that:

(1) Of 119 men, practically all Hawaiians or persons of mixed Hawaiian blood, living in the same house with the lepers, five, i.e., 4.2 per cent., developed leprosy.

(2) Of 106 women, practically all Hawaiians or persons of mixed Hawaiian blood, living in the same house with lepers, five, i.e., 4.7 per cent., developed leprosy.

(3) Of 12 women, all Caucasians, who lived in such contact with lepers as is necessary in administering to their bodily and spiritual wants, none developed the disease.

(4) Of 23 men living under the same conditions in contact with lepers, three, i.e., 13 per cent., developed the disease.

(5) The shortest period in which the disease developed after the person entered the settlement was three years (two cases), and the longest seventeen years.

In a report made in 1886 it is asserted that of 178 kokuas, 17 became lepers in a year. In a later report, made in 1888, it is remarked that of 66 kokuas examined 23 were found to have become lepers.

Such a condition of affairs does not exist at the present time at Molokai, and the change for the better is no doubt due to the great

improvements made in the sanitary conditions of the settlement in recent years.

Of course it should not be forgotten that, as the kokuas are drawn from a population in which leprosy is not very uncommon, it is quite possible that a certain number of cases came to the settlement in a stage of incubation.

Hollman examined carefully the conditions affecting the development of leprosy in the children of lepers at the Molokai Settlement, and came to the following conclusions:

(1) It is shown that 40 per cent. of the children born of parents of whom one or both were lepers died under one year.

(2) 32 per cent. of the males who were exposed ten or more years developed leprosy.

(3) 4 per cent. of the females whose average time of exposure was less than five years developed leprosy.

(4) 10 per cent. of the males exposed for more than seven years developed the disease.

(5) 13 per cent. of the females exposed from one to seventeen years, and under observation seven or more years, became lepers.

(6) The average time of exposure of the cases which developed leprosy was five years.

Accordingly, the danger of contracting leprosy for children born of leprous parents increases with the length of exposure.

Incidentally these investigations show that heredity does not play any important rôle in the causation of leprosy. Also, if it did, the disease would soon die out in any country.

The figures and conclusions show so clearly that contagion or infection through immediate contact is the usual mode of communication, that it appears rather far-fetched to seek an insect carrier of the scourge.

As a matter of fact, all experiments to prove this mode of transmission have so far failed, though it appears quite probable that the common house-fly can suck up the germs of the disease from open sores, carry them about for several days, and disseminate them in such a fashion.

The horrid sight of flies swarming and hovering over the purulent sores and round the nostrils of leprous beggars is well known to the traveller in eastern countries.



Graham-Smith, having shown that house-flies can harbour the bacteria of tuberculosis for twelve days or more, it seemed probable that the micro-organism of leprosy would show an equally long permanence in the fly-intestine.

Leboeuf examined numerous specimens of *Musca domestica* caught on the sores of lepers, in the wards, and in houses not further than 150 metres from the hospital.

He found leptotic 'globi' in the intestines of flies captured and kept for twenty-four hours, and acid-fast rods in flies thirty-six hours after feeding. His conclusions are that:

(1) *Musca domestica* can absorb enormous numbers of Hansen's 'bacilli' by nourishing itself on sores containing these germs.

(2) The 'bacilli' can be found in abundance and, apparently, excellent condition, in the excreta of the infected house-flies.

(3) It does not seem that multiplication takes place in the digestive tract of *Musca domestica*, but in any case there are no signs of degeneration.

(4) *Musca domestica* possibly plays an important part in the dissemination of leprosy by depositing its excrements on the mucous membranes or small abrasions of the skin of healthy people living in the immediate vicinity of lepers whose sores contain 'bacilli.'

It will be seen that in any case the fly does not do more than eventually disseminate the micro-organisms it has ingested, in a similar fashion to flies disseminating typhoid.

As far as our knowledge goes, no insect plays a real rôle of transmission in any bacterial disease. Moreover, transmission implies a more or less complicated developmental cycle in the body of the intermediate host, after which a protozoon can be inoculated by the proboscis of a biting or stinging insect. With bacteria a contaminatory communication through the faeces or by the regurgitation of the crop contents takes place. This is the case in bubonic plague or typhoid.

The fly is eminently adapted for a contaminatory or mechanical method of dissemination, but the difficulties inherent to the communication of leprosy to animals will render experimental work in this direction very difficult to accomplish.

It will be seen that the intimate personal contact, as found between a child and its mother, gives the most favourable circumstances for the acquisition of leprosy.

In South Africa, where the use of coloured and native servants and nurses for children is very widespread, it is not astonishing to find that about 40 to 50 per cent. of the white (Caucasian) patients under segregation cannot tell where they have contracted leprosy. Leprosy is quite widespread in the native population. In single instances, after a great deal of investigation, I was able to find out that the disease could be traced to a coloured nurse or servant who had evident signs of leprosy or eventually had been segregated in some asylum of the Union, the disease developing in the child many years afterwards.

Native labourers suffering from leprosy appear to be the source of contagion in many other cases. The Cape of Good Hope Leprosy Commission of 1895, having given careful attention to this matter, quotes several observations which support these views:

'An individual may be leprosy for many months, and it may be for a year or longer without showing outward signs of leprosy, and during that time may have communicated the disease to several without being aware of the fact that he is a leper himself. Hence arises the difficulty of knowing how and when the healthy may have been in contact with the contaminated. It is self-evident that the length of the period of incubation, and the possibility that during the period of incubation one individual may communicate the disease to another, greatly add to the difficulty of proving the source of contagion. Hence one may readily understand that statement of many lepers, that they had never been in close contact with a leper, or had never seen a leper, and consequently do not know how they got the disease. In many cases, however, possibly in by far the greater majority, it may be shown that at some period or other those who have acquired the disease have been in contact with a person or persons suffering from the disease; and even where direct contact cannot be proved, indirect means of acquiring leprosy, such as the use of the same bedding, or articles of clothing, or utensils, or a pipe, or implements used by a leper, or living in a room occupied by a leper, or attending to a leper, may be proved to have been present at some time or other in the life history of those who have contracted the disease.

In the evidence given before your Commission, Dr. A. J. J. Simons, of Malmesbury, who, in the course of an extensive practice reaching over a period of years, had many opportunities of observing the disease, gives a very instructive history of the course of leprosy on a certain farm in that division. In the case under consideration the leprosy was first noticed by him in a coloured man, a bastard Hottentot; next a little girl, the farmer's daughter got the disease; next another coloured man on the farm, a wagon driver who used to be great friends with the bastard Hottentot became diseased; after the wagon driver his master, the farmer, became leprosy; next a girl who came to the farm to attend the farmer's daughter and nurse her; and finally the farmer's niece who frequently visited the farm, spent the day there and was generally intimate with the leper daughter of the farmer. Everybody who is acquainted with the patriarchal

mode of life of the South African farmers, and who knows the habits of our rural population, will admit that the account given by Dr. Simons is a history typical of what happens in every country district in South Africa generally.'

Dr. Nieuwoudt, Darling, records the following cases: (1) A patient, in whose family, apparently, the disease was not present, was waited on by a leprosy servant, and developed the disease in a mixed form. Later on a boy who attended on him, etc., got the disease and died; later on the gentleman's daughter developed the disease in the tubercular form and is still here, though her father has since died. (2) An old man had the disease in whose family the disease did not exist, so far as known. His brother's son, who wore the same hat of his, got the disease; and another man outside this family, and of a clean family, in whose bed the patient slept, also developed the disease.'

Dr. Newnham, Aliwal, knows a case where a farmer was clearly infected by his native coachman who had been long a leper. Master and servant when travelling together used the same horn drinking cup.'

Dr. Vanes, M.L.A., of Humansdorp, writes: "In a family living at Patentie there were four children affected, the parents being healthy." With regard to these last cases it must be noted that many examples may be cited, which prove that children get the disease before the parents. Such cases appear to your Commission to plead powerfully in favour of the contagiousness of leprosy and as powerfully against its heredity. More especially in those cases in which no remote family taint can be shown.

Dr. Barry, of Bedford, quotes the case of a Dutch farmer, V.H., who states that he got the disease through handling a spade used by a leper Hottentot.'

These observations might possibly be set aside by the hypercritical if they did not agree in a striking fashion with the inferences deduced from the investigation of the spread of leprosy in many other parts of the world.

We know that at the present moment about twenty-five to fifty lepers or more are living in England: and yet of these only one has acquired the disease in the United Kingdom (mother and father were lepers). The simple precautions these unfortunates are able to take to keep themselves separated from their families have been sufficient to prevent contagion.

As a contrast we have India, where the last census appears to show an increase of lepers in the last ten years from 100,000 to 110,000.

The modern medical eye looks, therefore, upon leprosy as a disease which is definitely contagious, but to a very slight degree

under proper sanitary conditions. In situations where hygienic precautions are defective and the contact between the diseased and the healthy is unnecessarily immediate, where a leper is obliged to sleep in the same bed with other members of the family, and personal cleanliness apt to be in abeyance, the danger of contagion is certainly present to a markedly increased extent.

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## IX. EARLY AND DIFFERENTIAL DIAGNOSIS. PROGNOSIS

The early recognition of leprosy presents difficulties commensurate with its importance; for at times the symptoms may be so vague and indefinite that in the absence of conclusive microscopical evidence a correct diagnosis can only be made by a process of exclusion. Cases in which nodules containing numerous acid-fast rods, or in which typical maculae accompanied by anaesthesia or even contractures can be detected, cannot generally be considered as early conditions. Moreover, these ought to be diagnosable by someone who has a practical knowledge of the disease. It is in connection with slight and evanescent symptoms that serious difficulties arise, for such indefinite appearances are generally the only ones noticeable during the prodromal stage.

Lepers with very slight and atypical lesions are not necessarily early cases from the chronological point of view, because the slight areas of anaesthesia or discoloration may have existed unnoticed for years.

Sir Patrick Manson mentions the case of a gentleman who developed leprosy five years after noting in his diary a long series of headaches, transient fever attacks and progressive deterioration of health and vigour. These prodromal symptoms were followed three years afterwards by profuse perspiration which occurred without obvious cause. Such carefully observed cases are not frequent, and in view of the abnormal length of the period of incubation of leprosy it is often impossible for the patient to remember the occurrence of slight indefinite prodromal symptoms.

What in many cases is apparently the onset of the disease is but the more marked appearance of symptoms which have been present for a long time. Correspondingly very slight evidences of leprosy may be the traces of much more apparent previous pathological processes. It is accordingly often practically impossible to say whether we are dealing with an early case, though the symptoms may not be conclusive.

It should not be taken for granted that any case showing leprotic symptoms necessarily will progress and become irreco gnisable through deformities and mutilations because leprosy may get arrested in the incipient stage.

A disease with such multiform variety of clinical features is naturally often extremely difficult to recognise in its first stages, not only because of its tendency to produce symptoms which are not typical for any single condition and common to several ailments, but also because of the corresponding variety of initial appearances.

As a rule anaesthetic cases begin with a peculiar numbness in a digit, preferably the little finger of one or both hands. This numbness the patients compare to feeling that their fingers are covered with leather. On plunging the hand in warm water a peculiar sensation is noticed in so far as the finger or fingers affected feel the pain caused by the heat to a much lesser degree. The same symptoms may first show themselves in a little toe. On observation it may be seen that the fingers affected are glossier, more shiny than the corresponding or other fingers of the same hand. On taking exercise in warm weather it will be noticed that these areas are much less apt to perspire. Contemporary with these anaesthetic symptoms maculae or discoloration may be present or not in other parts of the body. Under some circumstances the maculae can be so slight

as to amount to little more than a peculiar discoloration very similar to *cutis marmorata* or mottled skin due to effects of cold, or they may take the shape of slight red lines which may form a closed figure. In course of time a case like this becomes a definite maculo-anaesthetic leper, or develops nodular features. A third alternative is that the symptoms may slowly but surely disappear, leaving hardly any trace.

The insensibility to heat and cold may be accompanied or heralded by formication and shooting neuralgic pains in the limbs affected. Profuse sweating precedes often the anhydrosis.

Pigmented spots may appear on various parts of the body, varying in colour from a light lemon yellow to a dark dirty brown. On looking at the spots under various angles of light incidence it will be noticed that the skin does not reflect the light everywhere in the same fashion, but is rougher in parts and appears covered with a slight farinaceous deposit which consists of desquamating superficial epithelial cells; the surface is intersected with very fine wrinkles, and has a dry atrophied appearance.

Another early stage consists in puffiness and infiltration round the *alae nasi*, or thickening of the lobes of the ears and loss of the eyebrows, accompanied often by intensive irritation and itching of the neck and back of the scalp of the head. In such instances epistaxis and dryness of the nostrils may be followed by a persistent cold in the nose.

A further early stage may be shown by a small pimple or blind boil, appearing in some part of the body, generally the face, this being followed by an outcrop of typical small nodules, superficial or subcutaneous. Under certain circumstances some sore or wound may be found to heal with extreme difficulty, or a slight abrasion may turn into a chronic ulcer; these have often been considered to form the initial sore or lesion of leprosy. They are at times, however, but a symptom of an infection which is already fully developed.

In dealing with such extremely early cases one should never fail to map out the extent and measure the intensity of the paraesthetic symptoms, remembering that in the beginning maculae are usually hyperaesthetic; pin-pricks, etc., are painful which under ordinary circumstances would cause hardly any discomfort; the sense of

touch can be unimpaired. Special value is to be laid on the faculty of recognising blunt and sharp (a penholder or a sharpened lead pencil will do for the purpose) and in each case a trial with two test tubes filled with warm (50°-60° C.) water, and cold water should be used to discover the patient's sensibility in distinguishing hot and cold in the affected areas. Slight muscular atrophy of the interossei should be looked for.

In all doubtful cases a microscopical examination of maculae or discolorations should be undertaken, though the interpretation of any histological lesions observed is a matter requiring expert knowledge. Needless to say, acid-fast rods should be searched for in the nasal mucous membrane; they may be present for a short time and then disappear for longer or shorter intervals.

Small nodules can be grouped up and treated with Anti-formin, centrifuged and the sediment examined for bundles of acid-fast rods. This method should, however, be only used in conjunction with an histological examination, because single acid-fast micro-organisms, without the lesions described elsewhere, are not sufficient to diagnose leprosy.

The fusiform thickening of the ulnar or other nerves, which is one of the cardinal symptoms of anaesthetic leprosy, as a rule does not come into practical consideration as a help in the early diagnosis of leprosy, because in the initial stages it is not usually present to a sufficient degree to render its detection easy. Moreover, such swellings are often deeply seated, and therefore not accessible in clinical examination.

#### DIFFERENTIAL DIAGNOSIS

Apart from the histological and serological features which have already been discussed, the differentiation of leprosy from other skin lesions, which are somewhat similar in appearance, is generally based on the assumption that leprosy is an extremely slow and intractable disease, and that its manifestations are essentially indolent in character.

Much has been written on the difficulty of distinguishing leprosy from syphilis. There can be no gainsaying that in very rare instances—a case has been illustrated by Graham Little—a

momentary confusion can arise, but a practical knowledge of both complaints soon shows that they must be quite different in their naked eye appearances.

Syphilis and yaws as a rule produce, in all stages, lesions which are more vascular, angrier, with a more active and proliferating appearance than leprosy, which is an eminently indolent, torpid, slow pathological process.

This difference is also applicable to the scars left by ulcers in these diseases. Syphilitic scars are usually rough, with numerous small fleshy warts and excrescences, and their margins are irregular with numerous small pockets and indentations. At times peculiar minute bridges of tissue can be observed under which a sound or needle can be passed. All these appearances are the result of a process of active proliferation and production of cicatricial tissue. Leprosy leaves scars which are smooth, shiny, and usually flush with or gradually merging into the surrounding healthy skin.

Accordingly any circinate macular efflorescences in syphilis and yaws should be more raised, redder, bleed more easily than a corresponding leprotic manifestation. In addition they should respond to mercury or salvarsan.

Practically every macular leper shows at least incapacity of distinguishing blunt from sharp and hot from cold (thermanesthesia) in some area of his body. Without this crucial test it is not advisable to diagnose leprosy, however typical the lesions may appear to the naked eye, unless the opinion can be confirmed by the microscope.

Leucoderma in the negro and dark-skinned races is often mistaken for leprosy. Here again the lack of anaesthetic or thermanaesthetic symptoms, the uniformity in hue and spread of the white spots, ought to facilitate a decision.

Lupus and tuberculosis of the skin are the pathological processes whose manifestations may cause considerable difficulty. However, lupus is rarely multiple, has an early tendency to break down and ulcerate, forming scars at its periphery. Lupus is more friable and bleeds less easily, and though more destructive than leprosy is usually somewhat more amenable to local treatment.

Pellagra ought not to be confused with leprosy, and *vice-versa*. Pellagra is symmetrical, strictly confined to parts generally exposed



to the action of the sun: there are no marked disturbances of sensibility in the areas affected, and the lesions are much more uniform and regular than is usually the case with lepra. The edges are not indented or irregular. In doubtful cases the microscope should decide.

The ringed circinate maculae seen in Caucasians in early stages of trypanosomiasis are not easily distinguished from some phases of early macular leprosy; in fact in one case to my knowledge the presence of the diagnosis was only cleared up by finding trypanosomes in the blood. Leprotic lesions are, however, more permanent and are not as fleeting as the similar eruption in trypanosome disease ('trypanides').

Syringomyelia, especially in its initial stages, is extremely difficult to distinguish from lepra, unless oculo-pupillar paralysis (consisting in myosis, diminution of the eyelid aperture and decreased prominence of the eyeball, Moritz) is present in the former eventuality, or typical maculae can be detected in the latter. A search for clumped acid-fast rods should be made. Early leprosy, moreover, does not often show such generalised lesions as syringomyelia.

In advanced cases, symptoms on the part of the medulla oblongata, scoliosis, and the absence or presence of thickened nerves, ought to help the diagnosis.

The absence or presence of thermanaesthetic and analgetic symptoms should be carefully investigated when dealing with coloured races, for numerous skin diseases due to fungi cause lesions which resemble leprosy in quite a disconcerting fashion. Here, again, microscopical evidence should not be missed.

In conclusion, it need only be added that in many instances the locality from which a patient comes, etc., the possibility or probability of contagion, are facts which, though they cannot carry conviction in every case, still may on the other hand simplify the diagnosis. It will be seen that very often recourse will have to be made to trained microscopical and bacteriological examination of excised fragments of skin.

The extreme variability of all the initial features of leprosy and the chronic course of the complaint, allow only a most guarded prognosis to be stated in any single case of the disease. It cannot

be denied that, given skilful medical attendance and healthy surroundings, even this most dreaded complaint can be held in check for comparatively long periods. It is true that little can be done for advanced cases, except from the standpoint of surgery. Early cases, however, will show but little impairment of their general health under favourable conditions and are quite capable of living a useful life for years and years.

From what has been said in connection with early diagnosis, it is evident that it is not safe to predict in the early stages what course leprosy is going to take; as a rule, cases which have begun with indefinite anaesthetic symptoms, last longer than those whose nodules have appeared in batches or have shown an extensive appearance of simultaneous patches.

The extensive mutilations of many anaesthetic lepers are but too often due to lack of attention to trifling casual wounds and burns.

#### **X. THE TREATMENT OF LEPROSY**

Not only nearly every imaginable drug, but in addition poisons and venoms, have been tried on lepers with the intention of discovering a cure, but this notwithstanding we have yet to find a prompt and sure method of treatment.

The present position can, however, be summed up in the words that even the most useful therapeutic substance still requires the help of an early diagnosis and proper hygienic conditions; but that at no time has the outlook regarding the treatment of leprosy been so hopeful as at the present moment.

In its initial stages leprosy affects the general well-being and appearance of an individual to such a slight extent that any therapeutic effort which succeeds in arresting the disease in its early stages in a fair proportion of cases would practically amount to a cure.

Spontaneous remissions of the disease, and spontaneous apparent 'cures' take place, however, in a small proportion of lepers, and can last for several years, in some instances as long as 15 years or more, after which the disease may again become virulent and rapidly carry its victim to the grave.

Therefore any drug or method of treatment must stand the test of time—five years or more—and have been carried out on a sufficient number of patients.

Without taking into consideration these important factors it is absolutely irresponsible to speak of cure in a chronic, slow, intractable malady such as leprosy.

A review of all therapeutic attempts would fill a volume by itself. I may, however, be allowed to give the results of the experiments made at Robben Island, and in South Africa generally.

For all advanced nodular stages Chaulmugra oil, or better still its refined constituent 'anti-leprol,' injected intra-muscularly 3-5 c.cm. at a time, is still the best palliative I know of. The injections should be repeated every three days and the course last five months or more if the patient can stand it, because at times the injections may become very painful. Chaulmugra oil and Antileprol can also be given internally in small capsules.

Antileprol is decidedly preferable for the latter mode of treatment, as it does not cause the gastric disturbances which are the outstanding feature of the unrefined oil. Doses varying between 15 m. and ten times that quantity can be taken daily.

Chaulmugra oil is a product of the seeds of the *Taraktogenos kurzii*, whilst the oil extracted from *Gynocardia odorata* ought to be called false chaulmugra (D. Hooper; Article on *Taraktogenos kurzii* in 'The Agricultural Ledger,' 1905, No. 5). It appears that substitution and adulteration of this oil is extensively practised, owing to the difficulty of keeping up a regular supply of the seeds of the *Taraktogenos kurzii*. Further particulars relative to this important subject are to be found in the paper by Hooper, which no doubt explains the variable results obtained in treating lepers with chaulmugra oils from different sources.

Brocq and Pomaret mix 70 parts of chaulmugra oil with 30 of eucalyptus oil, and find that the injections of this product are much less painful than the pure oil.

Early macular cases appear to respond better to other forms of treatment. A cultural extract has been prepared from Kedrowsky's isolation of the micro-organism of leprosy on similar lines to Koch's Alt-Tuberkulin. It contains accordingly the water-soluble toxic constituents of the bacillus, which, however, in the case of Hansen's

germ are very feeble indeed, so that much higher concentrations have to be injected than is the case in the analogous treatment of tuberculosis by tuberculine.

Dr. MacLeod of Charing Cross Hospital, and Dr. T. S. Davies, Resident Medical Officer of the Pretoria Asylum, have made use of this substance on single chosen cases. The results in both instances have been very satisfactory. The maculae or spots have slowly faded away.

Dr. MacLeod's patient, a young lady from the West Indies, has improved to such an extent that the disease is no more diagnosable. I am authorised to say that other therapeutic efforts failed to bring about any change for the better, and that the patient has now been observed for over two years without any remission taking place. On the contrary a further subjective improvement has so far taken place that muscular pains which were present in the arm affected have disappeared.

Dr. Sidney Davies's first patient, a little girl of  $8\frac{1}{2}$  years, was covered with dusky red patches all over her body. These faded away in the course of treatment extended over eighteen months, leaving only a slight discoloration (Pl. VI, fig. 6).

Later, six other lepers were injected with the same cultural extract at Pretoria, with the result that a remarkable improvement was brought about in two (this includes the patient already mentioned), marked improvement became noticeable in three, and slight improvement in the remaining two.

The injections are being continued, and at the time of writing further progress has been made, that is to say, that the maculae have continued fading and in some cases have disappeared, leaving only a trace of faint discoloration. Details will be published in due course.

It now remains to be seen whether these results will stand the test of time, and whether the same amelioration takes place in larger experimental series.

This method of treatment does not appear to be of real advantage in advanced nodular cases. The injection of one or two cubic centimetres of the cultural extract causes a rise in temperature which is accompanied by the appearance of bacilli in the blood. This may possibly lead to dissemination of the germs of the

disease. If the individual affected is young and otherwise healthy, and the leprotic symptoms not marked, a course of injections beginning with one cubic centimetre hypodermically every three days may be distinctly beneficial. The treatment should be continued for some time even after the maculae have disappeared.

The surgical treatment of leprosy requires most careful and constant attention. Fingers, toes, hands and feet are constantly getting septic, and prompt surgical intervention can really work marvels. Eye complaints, which are exceedingly frequent among lepers, are amenable to a certain extent to operative procedures, but relapses often follow even after the most skilful intervention.

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#### XI. PREVENTION, ASYLUMS AND HOME-SEGREGATION. SETTLEMENTS AND LEPER COLONIES

The trend of all recent research has been to prove conclusively that though leprosy is communicable from the diseased to the healthy by direct and indirect contact, still relatively simple precautions are sufficient to reduce the peril of infection to a considerable extent. Thus the probability of the scourge rapidly spreading and giving rise to an extensive epidemic is certainly remote. On the other hand, if hygienic precautions are neglected, the danger for the immediate surrounding and family of a leper is by no means to be despised, in view of the gravity of the disease.

The fortitude with which some nations view the slow but continuous increase of leprosy in their midst deserves to be explained, at least to obviate its being misunderstood for some less admirable virtue.

If the news spread that robbers or thugs or wild tribesmen were killing, torturing or maiming about five hundred or a thousand

people a year, I have no doubt that expeditionary corps, the police, and all the full weight of official indignation, would be employed to crush the criminals out of existence, and quite rightly so. On the other hand, an acid-fast micro-organism, against which we have hardly any chance of defending ourselves unless we are armed with the sharpest weapons of preventive medicine, is allowed to corrupt the bodies and souls of hundreds and thousands of new victims yearly without a single protest being heard. This is due to the fact that a biological mode of thought has not yet permeated the body politic, and that accordingly it is not yet sufficiently understood that public health means public wealth and individual health. We know of no other method of eradicating leprosy from a country than by segregation. All the critics of this method of dealing with the disease have no other practicable suggestion to make. It is true that by improving the hygienic conditions of the homes and lives of the poorer population, by carrying through an efficient and widespread sanitary inspection, leprosy could be slowly and definitely stamped out; but to deal with the scourge promptly nothing else can be suggested but isolation. The only argument that can be brought to bear against segregation is on the score of expense. In some instances parsimony and reluctance to invest money in public health matters have been veiled by the sentiment that it would be cruel to separate a leper from his family and his friends. Two facts are, however, overlooked. One is that when an individual develops leprosy in a severe and noticeable form, he need not trouble about his friends: they leave him of their own accord. The other is that consideration of the feelings of one may mean the misery of the whole family. In South Africa one leper infected in the course of time, directly and indirectly, sixteen other members of his family.

It is erroneous to believe that all lepers being segregated are compulsorily detained, however penurious may be the conditions offered by an asylum. Many are but too glad to find a refuge, a shelter from the dreadful conditions and inhuman circumstances under which they had to exist in the outer world.

The new introduction of a scheme of segregation requires careful preparation, because experience teaches us that the mere building of asylums is not sufficient to eradicate successfully the disease.

Efficient isolation presupposes adequate, skilful, sympathetic medical attendance in asylums, and when such is forthcoming many of the terrors of segregation disappear. Even in leprosy-ridden countries it is necessary for the medical profession to learn to diagnose leprosy, especially in its early stages. The public must be persuaded of the fact that there is no credit or advantage to be gained from having even only a few hundred or few thousand lepers hidden away in the midst of the community.

In Johannesburg a prominent political man had a leper for about ten years as his gardener; the boy was repeatedly diagnosed as a syphilitic. The mixed case of leprosy figured in Abraham's chapter on the disease in Allbutt and Rolleston's *System of Medicine*, page 682, toured South Africa with an operatic company, was laid up in Johannesburg with an acute leprotic attack, was visited and treated by a medical man who failed to recognise the malady. Numerous similar instances could be quoted, all showing that, for a system of segregation to succeed, it needs above all the intelligent co-operation of the general medical public. Without it all efforts are of little use.

There can be no doubt that segregation in asylums, if properly carried out, is in every case an expensive matter. No doubt it is more expensive still, in the long run, if adopted in an incomplete fashion, because the sum total eventually employed will have failed to bring about a corresponding diminution of leprosy.

The Union of South Africa is at the present moment spending quite £100,000 a year on its lepers (according to the estimates for 1913-14, £97,800), and is segregating certainly not more than half the number of lepers which are believed to exist in the territories of the Union. Under the circumstances the question arises whether these stupendous efforts and large sum of money would not be better employed in stamping out diseases which are more infective, such as typhoid or syphilis, and show a better chance of responding to treatment.

Unfortunately we are dealing with a subject which is but rarely, if ever, approached without a certain preconceived view and sentiment. The general public, on the one hand, considers leprosy to be extremely contagious, shuns and has an absolutely fanatic terror and horror of the disease. As a contrast, the patients them-

selves and their families cannot as a rule be persuaded to take the simplest measures to prevent an immediate spread of the scourge. In face of this dilemma nothing but segregation seems to meet the requirements of both parties.

An alternative to isolation in asylums is home segregation, which, however, as a rule, fails to have the desired effect if not aided by constant inspection, which may become so irksome that many prefer to take refuge in asylums. In South Africa it has not been a success, though in Norway it has been possible to introduce the system on a large scale.

When dealing with numerous lepers, such as are to be found among native populations, a system of segregation in asylums does not seem to be advisable on the score of efficiency, expense, and practicability. In these instances isolation in settlements with natural boundaries (a river, steep hills, on islands, etc.) appears to be by far the best solution. Wives can then accompany their husbands and the usual amenities of kraal-life (cattle, agriculture) need not be dispensed with. A central hospital for the infirm and blind, a dispensary for dressings and ambulatory medical attendance, a home for untainted children, should be provided, but the inhabitants of the settlement, whether lepers or healthy, would have to work for themselves; the only restriction placed on them is that they are not allowed to leave a certain district or province.

Amongst natives generally, especially in South Africa, pure maculo-anaesthetic leprosy is by far the commoner type of the disease. Many of these anaesthetic cases are by no means incapable of work; in fact, graduated exercise and manual labour is distinctly beneficial to their general good health. In asylums, strapping young natives, with a simple contracture or a few maculae, slide into a life of sloth, mope about the wards and yards, and if re-infection can take place, certainly expose themselves to its danger to an unnecessarily increased extent and jeopardise accordingly their slender but possible chance of recovery.

Asylums for lepers cannot be done away with; they are equally necessary, if not more so, than hospitals for the mentally afflicted. Where feasible, they should be supplemented by settlements or leper colonies, and in exceptional and carefully selected cases by home segregation.



By these means there is every reason to believe that leprosy would soon become extinct in any country, however a firm and extensive hold it may have taken, however unsuccessful previous half-hearted attempts of prevention may have been, however long the scourge may have existed in the past.

## XII. CONCLUSIONS

Though numerous questions are yet unanswered in the pathology of leprosy, and many points are still under discussion, it cannot be denied that the researches of the last ten or fifteen years have opened up further fields of fruitful investigation, and helped to elucidate several obscure problems.

It is true that the results of some experimental observations are so diametrically opposed that it appears hardly possible that both can be correct, at least not in their entirety. This is no new feature in the study of diseases whose investigation presents more than ordinary difficulties. Malaria is a good example. First of all bacteria were isolated time after time from patients, and it was sturdily maintained they were the etiological factor of the disease. Then the protozoa in the erythrocytes were stated to be artefacts by men eminent in various branches of medicine. Later on the plasmodia were found in marsh-water and so forth. Finally, the present conclusive standpoint was reached, but the path to truth was strewn with the thorns of controversy and deeply indented with the pit-falls of erroneous deductions.

This comparison explains the present position of leprosy research. The exercise of sound and reasoned scientific criticism ought to enable us to unravel, in course of time, the delicate and elusive thread of positive fact from the tangled skein of mistaken interpretations of essentially correct, but irrelevant observations.

The main conclusions which can be deduced from a perspective review of recent experimental study of leprosy appear to be concentrated in the following main points:

### *Cultivation of Hansen's 'bacillus.'*

Any micro-organism isolated from lepers which claims to correspond to the acid-fast rod seen in lesions should be identified

by injection into animals and the following production of the well-known histological features of lepromas.

The culture of Kedrowsky and those similar to this type are the only ones to fulfil this essential postulate.

*Clinical features of leprosy.*

In addition to the nodular, maculo-anaesthetic, and mixed types, a further variety is recognisable, with thick raised patches, which may be more or less confluent and circinate, containing numerous giant cells, and very scanty acid-fast rods.

*Histological appearances of typical leprotic lesions.*

In dermal nodules and corresponding lepromas of inner organs, there occur numerous matted masses of acid-fast rods, intra-cellularly and extra-cellularly situated, very slight tissue reaction and scanty giant cells.

The lesions of inner organs which show caseation, necrosis or numerous giant cells of the Langhans type, appear in some cases to be due to tuberculous complications. Guinea-pig inoculations only can conclusively decide the nature of such appearances.

Maculae show the features of slight chronic irritation due to the action of minute doses of bacterial toxins. This, in addition to the external resemblance between maculae and the erythema produced by injecting the water-soluble contents of various acid-fast cultures into lepers, seems to postulate that leprotic maculae may be the result of a local reaction similar to the one resulting in von Pirquet's and similar tests for tuberculosis.

*Rat leprosy.*

This spontaneous disease presents numerous points of resemblance to leprosy, and may yet be found to be etiologically related to the human malady.

Its experimental transmission in rodents only causes analogous but not identical effects to the spontaneous disease.

*Transmission to animals.*

The negative results of numerous experimental inoculations of human beings, and the relative low infectivity of leprosy, should prepare us to face very numerous unsuccessful results in dealing

with this question. It is only by injecting a large series of animals in various ways, and then observing them for prolonged periods, that deposits of acid-fast 'bacilli' can be produced in the inner organs which have the essential histological features of the leproma.

#### *Serology.*

Wasserman's test is incapable of distinguishing in every case syphilis from leprosy. The same applies to Eitner's test. Noguchi's luetin test is negative in lepers whose disease is not complicated by syphilis.

Leprotic sera often show spontaneous absorption of complement. The serological distinction of leprosy and tuberculosis is not possible in every case. Tuberculine tests may be present in lepers showing no clinical symptoms of tuberculosis.

Agglutination is so far of little value owing to self-clumping of acid-fast bacteria and low titer of leper sera.

#### *Contagiousness.*

The communicability of leprosy by direct and indirect contact, especially under defective hygienic conditions, has been established by numerous repeated and independent clinical observations.

#### *Treatment.*

Leprosy, especially the macular variety, is subject to spontaneous remissions and self-cures.

Chaulmugra oil is indicated for nodular cases; cultural extract for macular lepers (as far as the present experience goes).

#### *Prevention.*

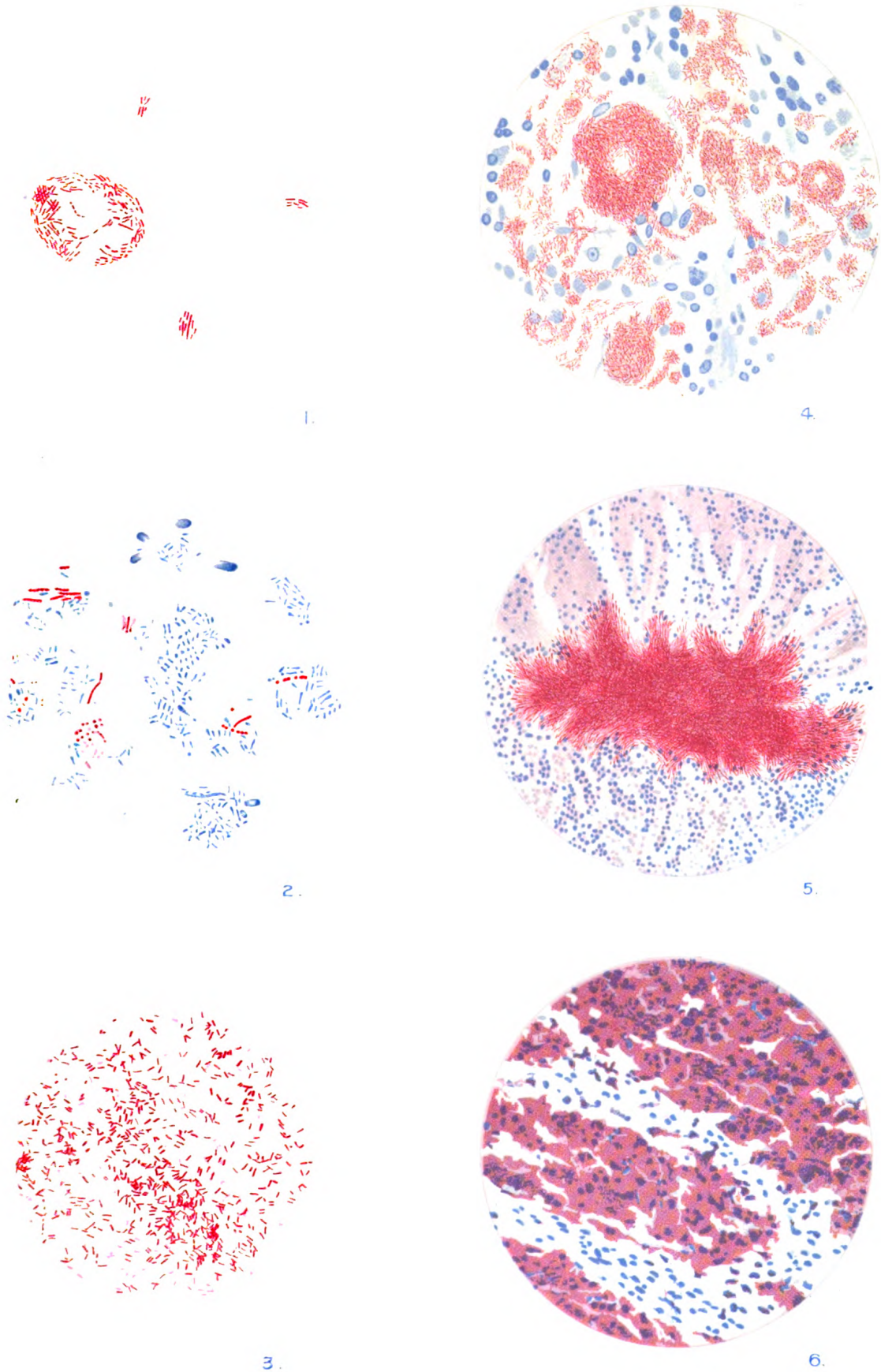
Registration and sanitary inspection of lepers' habitations; segregation in asylums, settlements, or colonies; early separation of children from leprous parents; betterment of hygienic conditions where leprosy has spread extensively.

## EXPLANATION OF PLATES I-VI

## PLATE I

- Fig. 1. Filamentary interlacing, branching, non-acid-fast germ cultivated from the nodule of a leper on horse-serum-nutrose-agar. Some of the original acid-fast rods from the leprous lesion are still to be seen, and the acid-labile bacteria have grouped themselves in a peculiar fashion round the 'globi.' This culture was injected into a rat, acquired acid-fast properties, and could be then regained in pure culture as an acid-fast 'bacillus.' Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.
- Fig. 2. Diphtheroid, pleomorphic, filamentary and bacillary germ, isolated from the nodules of a leper. It is considered to be a further developmental stage of the previous micro-organism. These bacteria are slightly acid-resisting, and regained acid-fast properties after injection into animals. Third sub-culture on placental-juice-glycerine-agar. A slight multiplication of the original acid-fast rods may have taken place. Stain: Ziehl-Neelsen. Magnification, 1,300 diameters.
- Fig. 3. Acid-fast stage of the bacteria of leprosy which are commonly met with in tissues. ('Hansen's bacillus,' *Mycobacterium leprae*.) Pure culture with which all identification experiments have been carried out. Two months' culture at 37°C. on fish-juice-glycerine-agar. From a micro-photograph. Stain: Ziehl-Neelsen. Magnification, 500 diameters.
- Fig. 4. Section of omental gland from a spontaneously leprous rat, found in Ipswich by Drs. Petrie and Macalister. The similarity to the lesions seen in human leprosy is very striking. Stain: Ziehl-Neelsen. Magnification, 500 diameters.
- Fig. 5. Nodules produced in the kidney of a rabbit by the intravenous injection of a culture of 'Moeller's smegma bacillus.' This lesion simulates leprosy in one detail only: the presence of massed acid-fast micro-organisms. Stain: Ziehl-Neelsen. Magnification, 500 diameters.
- Fig. 6. Section from the skin of a leprous rat, showing the enormous quantities of micro-organisms which cause the lesions. The thick, purple masses consist almost entirely of 'bacilli'; the blue spots are the nuclei of the connective tissue.
- From a colour-photograph taken by Sanger-Sheppard's method. Stain: Gram. Magnification, 400 diameters.

[Plates I-IV have already been published by the author in the *S. Afr. Med. Record* (1913), XI, pp. 201-222.]



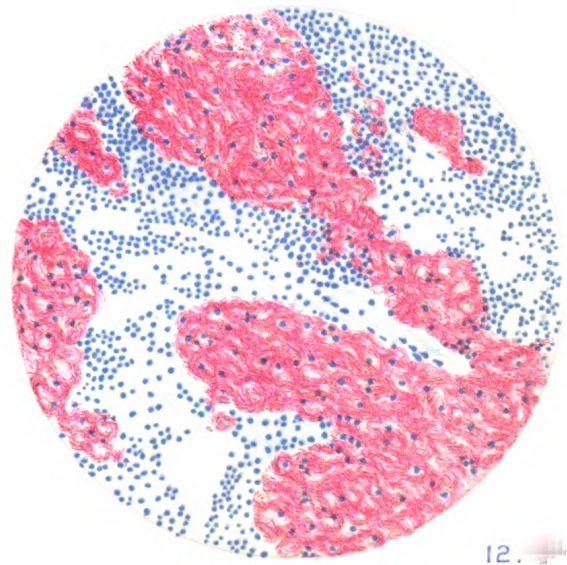
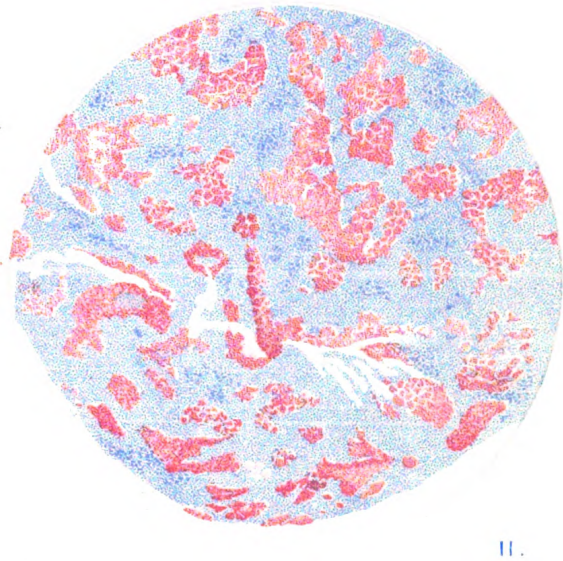
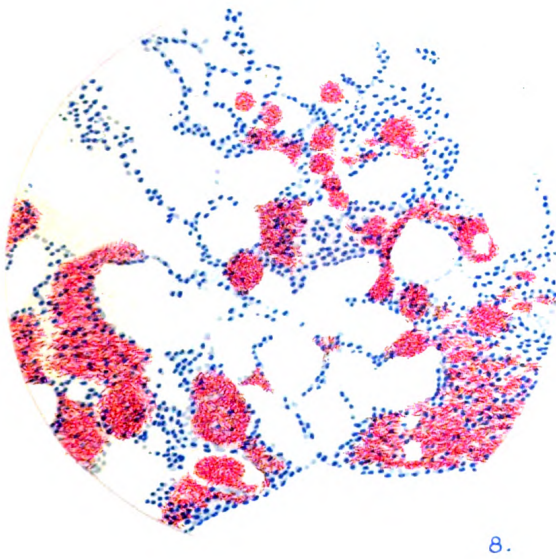
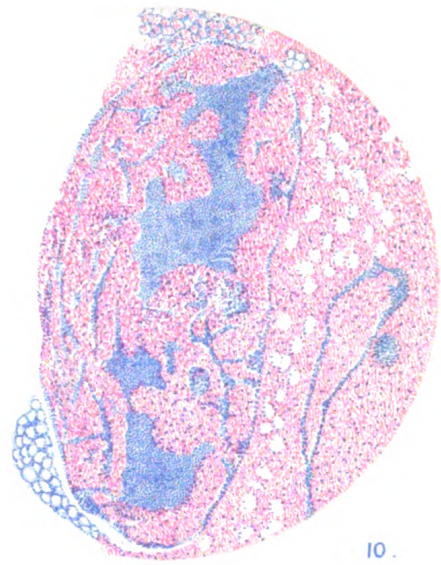
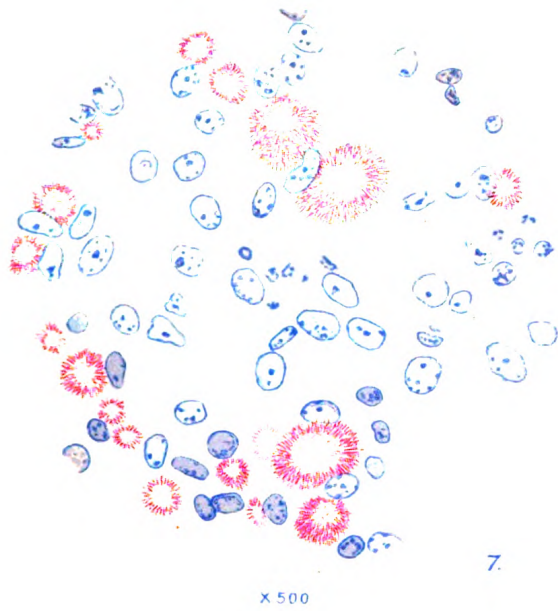




## PLATE II

- Fig. 7. Lesions produced in the liver of a rabbit after intravenous injection of the acid-fast culture of 'Hansen's bacillus.' Similar appearances can be brought about by the intravenous injection of cultures of human and avian tuberculosis, and under circumstances by the inoculation of leprosy 'virus.' Stain: Ziehl-Neelsen. Magnification, 500 diameters.
- Fig. 8. Lung of a mouse which has been injected intraperitoneally with Kedrowsky's strain of leprosy. The enormous quantity of 'bacilli' present has caused no appreciable tissue reaction of a tuberculous nature. Stain: Ziehl-Neelsen. Magnification, 150 diameters.
- Fig. 9. Spleen of a monkey injected intraperitoneally with Kedrowsky's strain of leprosy. Also in this instance the numerous bacteria have not brought about a tissue reaction of a tuberculous nature. Stain: Ziehl-Neelsen. Magnification, 35 diameters.
- Fig. 10. Omental gland of a rabbit, which received an intravenous injection of Kedrowsky's strain of leprosy. The section is teeming with acid-fast 'bacilli' which have not caused the death of the cells they have invaded, because the nuclei are still capable of staining, and have not appreciably altered in morphology. Stain: Ziehl-Neelsen. Magnification, 25 diameters.
- Fig. 11. Spleen of a mouse which had been injected intraperitoneally with an emulsion of Kedrowsky's strain of leprosy. The red patches are groups of cells which are crammed with acid-fast rods. Similar lesions are sometimes seen in the spleen of lepers. Stain: Ziehl-Neelsen. Magnification, 25 diameters.
- Fig. 12. Portion of the mouse spleen shown in fig. 11, but under increased magnification. The 'bacilli' are seen to have nearly completely filled the protoplasm of the cells without inducing necrosis or caseation. No giant cells of the Langhans type are to be detected. These are the points which on the whole distinguish this lesion from one of 'tubercular' nature, i.e., caused by Koch's 'bacillus.' Stain: Ziehl-Neelsen. Magnification, 230 diameters.









## PLATE III

- Fig. 13. Localised lesion produced in the testes of a rat by the injection of human leper 'virus.' Stain : Ziehl-Neelsen. Magnification, 500 diameters.
- Fig. 14. Leprous lesions in the spleen of a rat six months after injection of a ground-up inguinal gland from the preceding animal. Drawing made from three different portions of the slide. The peculiar hyaline increase in size of protoplasm of the cells invaded by the 'bacilli' is specially noticeable. Stain : Ziehl-Neelsen. Magnification, 500 diameters.
- Fig. 15. Section through the skin of a rat suffering from spontaneous rat leprosy. The acid-fast 'bacilli' of the disease have congregated round the shaft of a hair-follicle. (According to Borel may have been deposited there by *Demodex*.) Stain : Ziehl-Neelsen. Magnification, 35 diameters.
- Fig. 16. Small, solitary deposit of 'bacilli' in the omentum of a rat, one month after intraperitoneal injection with ground-up 'virus' from a spontaneously infected rat. The micro-organisms are markedly granular, yet juice of the spleen of this animal produced a pure culture of the germ, the only one achieved out of very numerous attempts. Stain : Ziehl-Neelsen. Magnification, 700 diameters.
- Fig. 17. Thorax gland of a white rat injected intraperitoneally with an artificial culture of rat leprosy. The lesion is identical with that produced by the injection of 'virus.' Stain : Ziehl-Neelsen. Magnification, 500 diameters.
- Fig. 18. Small deposit of acid-fast micro-organisms detected in a nodule of the abdomen of a mouse injected with an emulsion of a leprosy culture which had been heated for an hour at 60°C. Showing that, killed or alive, leprosy micro-organisms are capable of producing similar lesions. Stain : Ziehl-Neelsen. Magnification, 300 diameters.



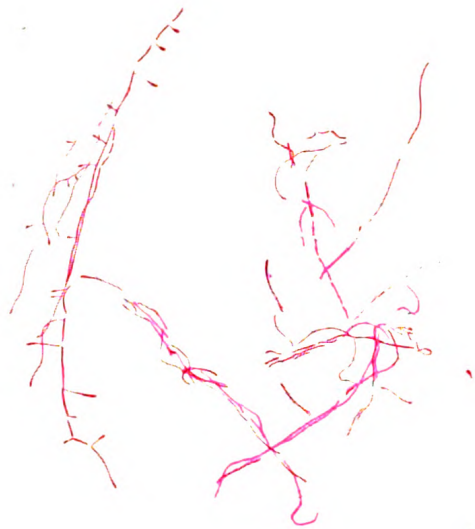




## PLATE IV

- Fig. 19. Filamentary, interlacing, branching, partially acid-fast and acid-resisting germ isolated from the pleura of a man, by Birt and Leishman. It will be seen that it shows marked clubbed fragments (so-called involution forms), and also some which are definitely 'bacillary' in appearance. The resistance to the bleaching properties of mineral acids varies also considerably. From a pure culture on glucose-agar. Stain: Ziehl-Neelsen. Magnification, 800 diameters.
- Fig. 20. Acid-fast bacteria from the water-tap at the Lister Institute. Here also marked pleomorphism and variable acid-fastness is noticeable, but in this case the culture is not known to be pure, because the micro-organism was not isolated on artificial media. Similar acid-fast rods have been found in the scraping from a water-tap in the Public Health Laboratory at Cape Town and in Stockholm; also in mule's dung at Robben Island. These observations show the ubiquity of acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 600 diameters.
- Fig. 21. Section marked 'Experimental Leprosy, Monkey,' sent by Professor Duval, and considered by this author to be diagnosable as leprosy. Stain: Haematoxylin-Eosin. Magnification, 60 diameters.
- Fig. 22. True branching in tubercle-bacillus from sputum of consumptive. From a drawing by Mr. W. D. Severn. Stain: Ziehl-Neelsen. Magnification, 2,250 diameters.
- Fig. 23. Pure culture of Duval's strain of leprosy. Its coccoid appearance shows it to be morphologically very similar to the ubiquitous group of saprophytic acid-fast micro-organisms, therefore quite different from 'Hansen's bacillus.' From a micro-photograph. Stain: Ziehl-Neelsen. Magnification, 1,100 diameters.
- Fig. 24. Mononuclear cells from the peritoneal cavity of a guinea-pig, forty-eight hours after injection with a chromogenic strain of an acid-fast micro-organism. (Duval's 'lepra.') Stain: Ziehl-Neelsen. Magnification, 1,800 diameters.

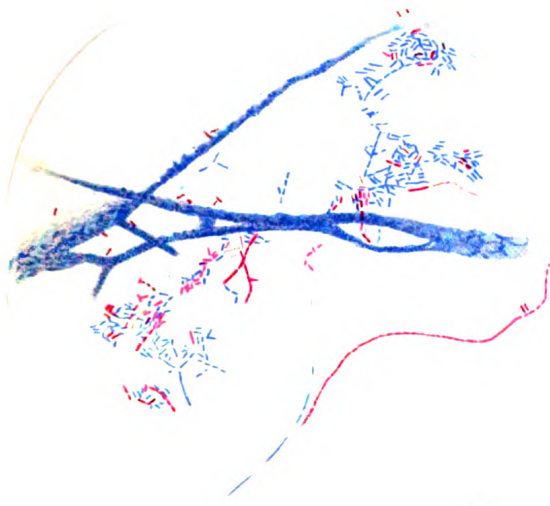




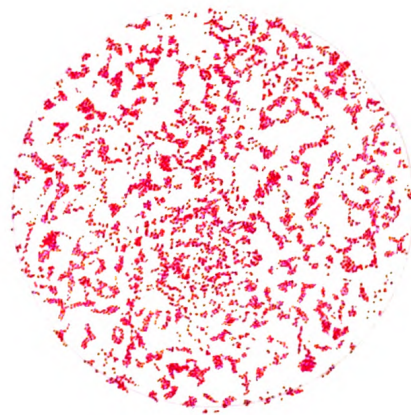
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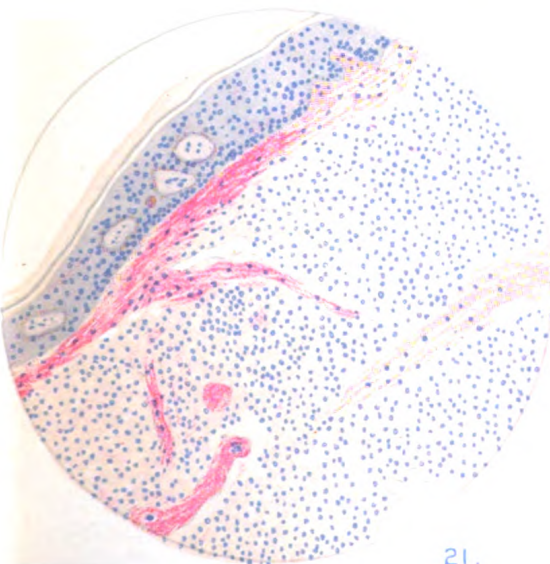
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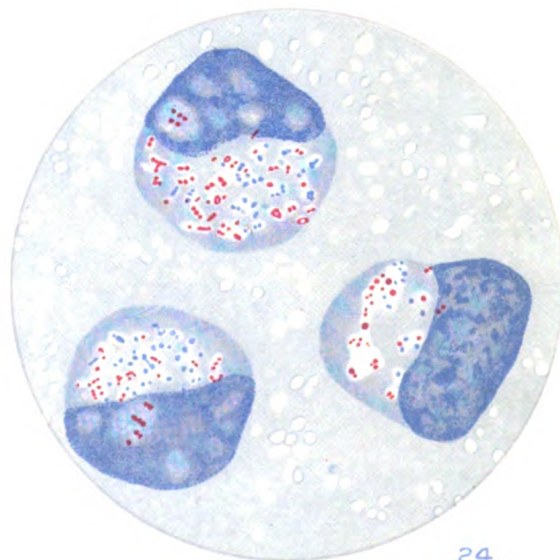
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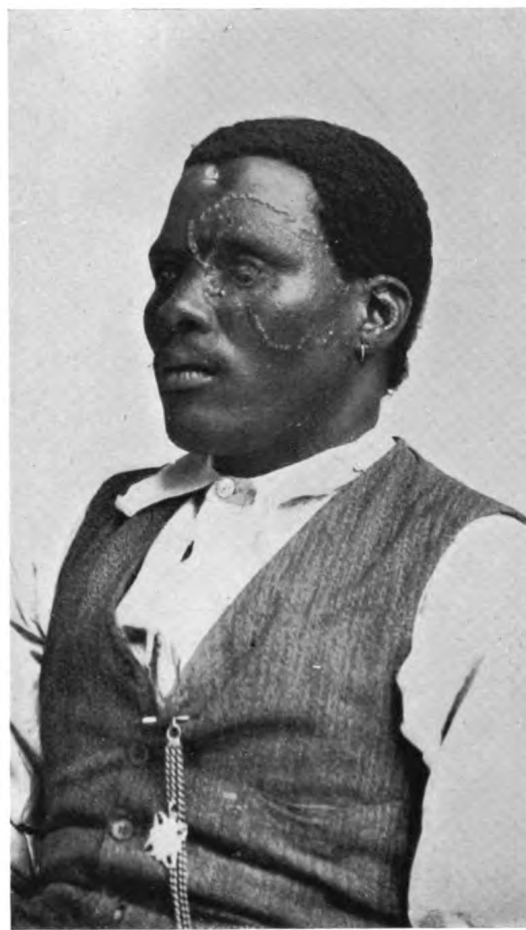
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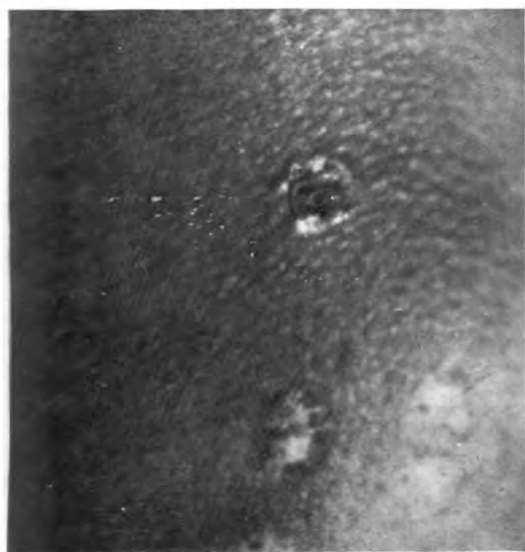
## PLATE V

- Fig. 1. Front and back view of an off-coloured Cape-boy, with serpiginous or lupoid leprosy. The presence of nerve-lesions and similar appearances on single other cases of leprosy, allow the inference that the disease is due to an infection through Hansen's 'bacillus,' though acid-fast micro-organisms are very scarce indeed in the skin-eruption.
- Fig. 2. Leprous patch of face, apparently taking its origin from the eye, in a Fingo native. The borders are more raised and the surface rougher than is usually the case in leprosy. Similar patches are present on the loins of this case. No anaesthesia or paraesthesia detectable.
- Fig. 3. Arm of nodular leper 24 hours after intradermal injection of 10 minims of cultural extract. There is a copper-coloured areola round the site of injection, in the centre of which a small sore has developed.
- Fig. 4. Arm of an arrested anaesthetic leper, 24 hours after intradermal injection of 10 minims of cultural extract. Numerous blisters; intense local reaction, rise of temperature to 101° F., malaise, pains. All symptoms passed within three days.



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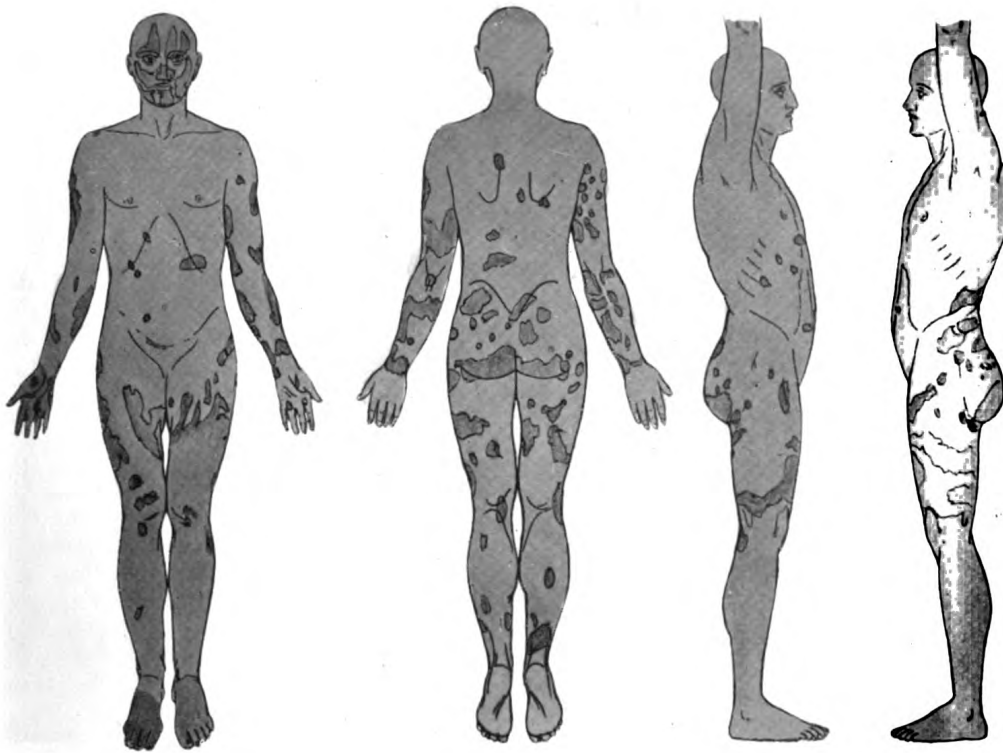




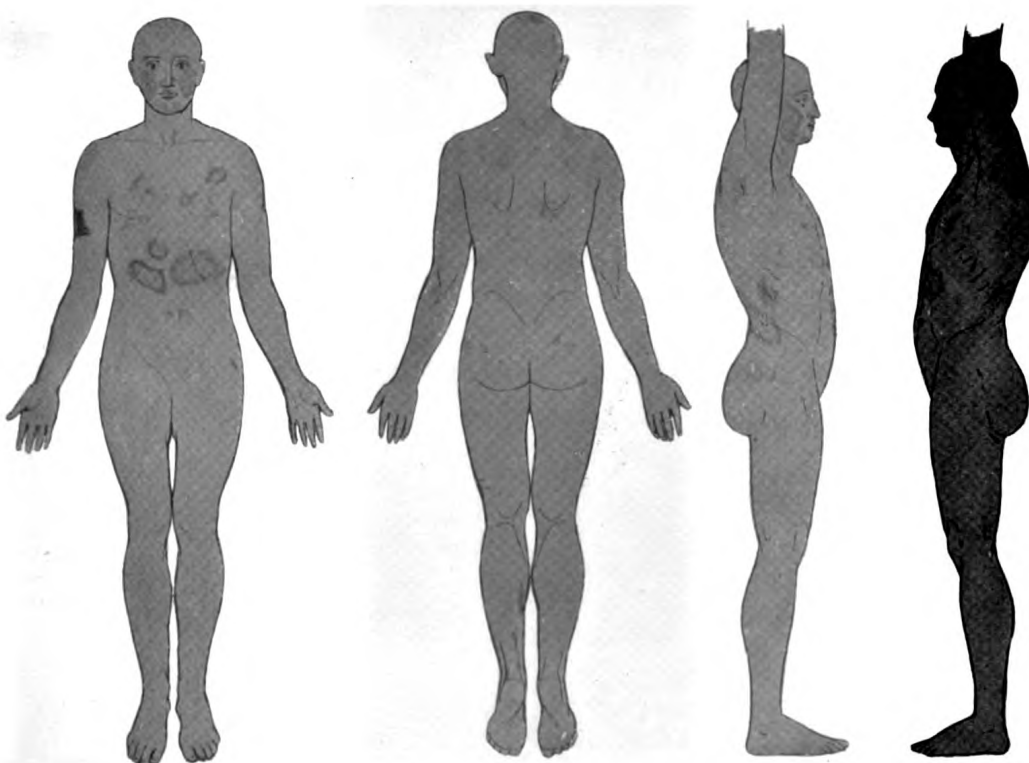
## PLATE VI

- Fig. 5. Diagrams of case before treatment (12.9.12). On face, infiltrated and thickened patches, red in colour. On legs, erythematous patches, reddish in colour. Over feet, purplish flush.
- Fig. 6. Diagrams of case after treatment (6.5.14). There are faintly pinkish-red flat discolorations on the face. There is a flat brown macula on right upper arm, below the shoulder. The other yellow discolorations are very difficult to chart, as many of the edges are not well defined. There is a faintly light purple flush on the limbs, and the insides of the hands are a darker shade of the same.





5



6



# THE METABOLISM OF WHITE RACES LIVING IN THE TROPICS

## I.—THE PROTEIN METABOLISM

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The effect of high temperature and a moist atmosphere upon the metabolism of the human body has been studied by workers in temperate climates, the conditions being artificially produced in a specially constructed chamber, and also by a few investigators who carried out experiments upon both acclimatised whites and natives living in the Tropics. In almost every case attention has been given to the total exchanges of the body, with the object of ascertaining whether the altered conditions bring about the introduction of any new factor into the mechanisms by which the body regulates its thermal equilibrium.

The most important investigations done in the Tropics were those of Eijkman in 1893, who determined the calorific value of the everyday food of a number of white men living in Java, and found that the average did not differ from that usually accepted as a standard in Europe. Examination of the food of Malays also showed that the heat value per kilogram of body weight was not materially different from that of the white men. In these researches balance sheets were drawn up between the total nitrogen taken in and that excreted, but no investigations were made into the separate nitrogenous products of protein metabolism.

A number of analyses of the nitrogenous constituents of the urine were made by McCay (1908) in India, but were confined to an examination of the urines of Bengalis, a people who conform to a diet with much lower protein content than Europeans.

In the course of a study of the effect of residence in tropical countries upon a working white race, it was thought that some information might be obtained by a fairly complete examination of the partition of the nitrogen and sulphur excreted in the urine. The significance of the different excretory substances in urine is but very imperfectly understood, and the effect of any departure from normal conditions is of interest from the possibility of throwing light upon the processes which constitute the nitrogenous metabolism of the body.

The principal product by which proteins give up their nitrogen in the body is urea, and the bulk of this substance appearing in the urine represents the ammonia produced by deamination of nitrogenous foodstuffs. It tells us nothing as to what has happened to the remainder of the molecule from which it has been derived.

Certain substances, however, appear to be produced by the metabolism of the tissues, the endogenous metabolism of the body. Thus, the creatinine is almost entirely derived from this source, since the quantity of creatinine excreted on a low protein diet is practically the same as when the diet is rich in nitrogen (Folin). Only a small fraction is due to creatine taken in with the food. Leathes (1907) found that during fever, when the tissue metabolism is known to be increased, the quantity of creatinine in the urine was also increased, and recent experiments on rabbits by Meyers and Volovic (1913) have confirmed this observation, and have shown that the output of creatinine is increased whether the pyrexia be caused by infection or by confining the animal in a hot atmosphere.

The total quantity of sulphur contained in the urine normally runs parallel to nitrogen, varying with the quantity and nature of the protein catabolised. The bulk of the sulphur is excreted in the fully oxidized form as inorganic sulphates, together with a small quantity in combination with indol and skatol as ethereal sulphates, these products being the result of bacterial decomposition in the intestine. A small quantity of sulphur is excreted in an unoxidized form, termed neutral sulphur, which in all probability consists mainly of cystine and thiocyanates. The output of neutral sulphur in normal individuals was found by Folin to be independent of the quantity of protein in the diet, and for this reason he considered

this portion of the sulphur as derived, like the creatinine, from endogenous sources.

From the experiments on creatinine mentioned above, it seemed possible that tissue metabolism might take place to a greater extent in a tropical climate, and it was of special interest to find whether the quantity of the substances derived from this source was very different from the averages in urines of residents in temperate climates.

Fairly complete analyses were made of the daily urine of four male subjects ranging in age from 25 to 36 years, and who had resided in Tropical Queensland from one to four years. The experiments extended in most cases over a week, the total urine being collected and each twenty four hours' sample analysed. The subjects were living an ordinary laboratory life, were allowed to choose their own diet, as the aim was to make the observations under the conditions of every day life. Moreover, most investigators are agreed that the knowledge of the diet being controlled is not without influence on the quantity of food taken, whilst if a fixed diet be partaken, the continued sameness affects the appetite. As a preliminary, therefore, the diets were uncontrolled as regards quantity and character, excepting in one case (protocol IV) where the subject was taking a milk and bread diet of known nitrogen content.

In addition to these four experiments, determinations have been made at different times of the total nitrogen and the creatinine in the daily urine of five subjects, and in two cases comparisons have been made of the quantities of these substances excreted during the hot rainy season and the cooler dry season.

#### METHODS

The total nitrogen was determined by Kjeldahl, and urea by Folin's (1912) potassium acetate method, uric acid by the Folin-Schafer method and the purin bases were estimated after precipitating the uric acid as recommended by Kennaway (1909).

For the estimation of creatinine Folin's well-known method was employed, but some modification was required on account of the high temperature of the laboratory. Although numerous papers

have been published on the conditions under which this determination should be carried out, such as the quantities of reacting fluids, the time during which the solutions are allowed to react, the temperature and the extent of dilution before the readings are made, all such conditions have been determined for room temperatures about 15-20° C. In this laboratory, during the hot season, the air temperature during the day is rarely below 27° C. and generally ranges from 30 to 35°, whilst that of the water supply is usually about 30°. The conditions laid down by other workers were, therefore, not applicable, and had to be readjusted to meet the requirements. For this purpose a solution containing 10 mgms. of creatinine in the form of the picrate (mpt. 205-206°) in 20 c.c. was employed, and the depth of colour produced by the addition of alkali and picric acid compared with a 0.1N solution of potassium bichromate in the Dubosq colorimeter in the usual manner. Experiments were also carried out with urine. It is only necessary here to give the conditions established, without going into the experimental details. It was found that at temperatures between 27° and 35° C., 10 mgms. of creatinine gave the maximum coloration within four minutes, whilst the colour faded if the reacting liquids were allowed to stand for longer than six minutes. The best quantities of the reagents were 15 c.c. picric acid (saturated solution) and 5 c.c. of 10 % sodium hydroxide, the whole being diluted to 500 c.c. as recommended by Folin. Within limits the total volume of the reacting fluids did not influence the colour, thus 5 c.c. of urine diluted either to 10 or 20 c.c. gave the same reading. The colour of the diluted mixture was found to fade fairly quickly, the solutions were therefore compared with the standard immediately after the final dilution was made. Most of the urines examined were very concentrated, and 5 c.c. usually contained enough creatinine to give a reading on the colorimeter between 7 and 9, the standard being set at 8. If, however, the reading came beyond these limits the estimation was always repeated with a larger or smaller quantity of the urine. The usual 0.1 N potassium bichromate solution was employed as a standard, and was carefully checked by a solution of pure creatinine picrate.

Of the other constituents the total sulphur was estimated by Benedict's method, the inorganic, ethereal and neutral sulphates by

Folin's methods, the phosphoric acid by titration with uranium acetate, and the chlorine by Volhard's method. The gravity was determined by Mohr's balance.

The full details of the analyses are given in the protocols at the end of the paper.

#### VOLUME, SPECIFIC GRAVITY AND TOTAL NITROGEN

Determinations of these have been made on the twenty-four hours' urine at different times, each experiment lasting from four to ten days so as to obtain a fair average. Most of these were done in the hot season of the year (November to April) when the atmospheric humidity is high, but in two cases comparisons were made in the cooler season (May to October).

The full details are given in the protocols, whilst the averages for each set of experiments are collected in Table 1, the number in

TABLE 1.—Volume, Specific Gravity and Total Nitrogen of the Urine

No.	Subject	Age	Weight kilos	Protocol	Season	24 HOURS URINE				
						Vol. cc.s.	Spec. grav.	NITROGEN IN GRAMS		
								Total	Per kilo of body weight	
1	A	32	66.8	1	hot	986	1.023	11.69	0.175	
2	A	33	66.8	2	hot	772	1.027	11.58	0.173	
3	A	33	66.8	3	hot	1,070	1.024	14.33	0.214	
4	B	25	60.6	5	hot	922	1.027	12.41	0.205	
5	D	30	63.1	8	hot	782	1.026	10.83	0.172	
6	D	30	62.8	7	cool	1,437	1.017	12.11	0.193	
7	E	36	48.2	11	hot	621	1.023	8.83	0.183	
8	E	35	48.4	9	cool	1,037	1.021	11.69	2.242	
9	E	36	48.2	10	cool	1,372	1.012	10.52	0.218	
10	C	27	62.2	6	cool	1,367	1.014	11.02	0.177	
Average for both seasons, 5 persons						=	1,070	1.021	11.49	0.194
Average for hot season, 4 persons						=	817	1.025	11.15	0.188
Eijkman's average for 19 Europeans in Java						=	1,442	1.017	13.04	0.200
Pflüger and Bohland for Europeans						=	—	—	12.67	0.194
Bleibtreu and Bohland for Europeans						=	—	—	14.93	0.233

the fifth column referring to the protocol containing the full data. For comparison are also given the figures obtained by Eijkman for Europeans living in Java, as well as those for Europe found by Pflüger and Bohland and Bleibtreu and Bohland. The volume of urine varies from day to day, but the average figures show that during the hot humid weather, when any exertion is accompanied by profuse sweating, the urines are comparatively small in volume and of high gravity. The volume of liquid taken in was generally between 3 and 4 litres a day.

Nos. 5, 6, 7, 8 and 9 show the effect of the season on the excretions of the same individuals. The total nitrogen also shows daily variations, but the averages, 8·8 to 14·3 grams, are somewhat lower than that given in the physiological text-books as the average for Europe, 14 to 18 grams. When the nitrogen is considered in relation to the body weight it does not differ appreciably from that found in Europe. In the cases examined the daily excretion of nitrogen was greater in the cool season than in the hot. Part of this difference is no doubt due to the nitrogen lost in the sweat during the hot weather. Eijkman (1893) found that as much as 0·76 to 1·36 grams of nitrogen per day were excreted cutaneously by Malays, whilst Benedict (1906) outside the tropics, found that during rest the nitrogen lost in this way was about 0·071 grams per day, but during severe muscular labour as much as 0·13 to 0·22 grams per hour.

During the hot part of the year a nitrogen-balance experiment was carried out on subject A. The numbers, Table 2, have not

TABLE 2.—Nitrogen balance on fixed diet.

Date	Nitrogen in food	NITROGEN EXCRETED			Balance
		Urine	Faeces	Total	
4.3.14	26·58	17·01	2·99	20·00	+ 6·58
5.3.14	26·58	18·87	1·50	20·37	+ 6·21
6.3.14	26·58	19·24	3·52	22·76	+ 3·82
7.3.14	26·58	20·32	1·82	22·14	+ 4·44
				Total ... ..	+21·05



been included in the general averages, since the diet, which was restricted to milk and bread and butter, was of much higher nitrogen content than the usual diet. The subject increased in weight by 1·3 kilos during the four days, and complained of feeling uncomfortably full after the meals. The numbers show a considerable nitrogen retention amounting to 21 grams, a small part of which may be accounted for by the cutaneous excretion.

#### UREA

The excretion of urea varies with the total nitrogen.

The average daily outputs in the different subjects varied from 26·98 grams in A to 14·79 grams in E, or 87·9 % to 78·4 % of the total nitrogen excreted respectively. The quantity of urea generally quoted as the average for Europeans on a mixed diet is 30 grams per diem; the quantities found were always less than this amount, but when considered in relation to the total nitrogen appear quite normal.

#### AMMONIA

The ammonia present in the urines was quite normal in amount, the averages varying from 0·50 grams to 0·64 grams, or 4·8 % and 3·6 % of the total nitrogen respectively. The average output for all four subjects being 0·57 grams.

#### CREATININE AND CREATINE

Creatine was only present in any of the urines on one occasion, and then only in a very small amount (0·12 grams nitrogen).

A number of determinations of the creatinine have been made both in the hot weather and in the cooler season. Table 3 gives the averages over the whole periods, given in detail in the protocols, each figure representing the average output per day for a period of several days. These averages vary between 0·47 grams and 0·71 grams of nitrogen per day, representing between 3·2 and 6·1 % of the total nitrogen. In normal individuals in temperate climates the daily excretion is, according to Folin (1905), from 3·5 to 4·5 %, whilst van Hoogenhuyze and Verploegh (1905) found values of 4 to 6 %. Leathes observed that although during fever there was an increase in the actual amount of creatinine excreted,

there was at the same time increased nitrogen, so that the percentage of the nitrogen which appeared as creatinine was actually diminished.

If the creatinine in the urine be considered in relation to the body weight, column 8, it is seen that the quantity excreted varies in the different individuals from 8.52 mgrms. to 10.62 mgrms. per kilo of body weight, values which agree with the figures given by Folin, namely, 7 to 11 mgrms. per kilo of body weight.

TABLE 3.—The Excretion of Creatinine

No.	Subject	Season	Protocol	Total N. grms.	CREATININE NITROGEN		
					grms.	per cent. of total N.	per kilo of body wt. mgrms.
1	A	hot	1	11.69	0.61	5.4	9.13
2	A	hot	2	11.58	0.71	6.1	10.62
3	A	hot	3	14.33	0.62	4.4	9.28
4*	A	hot	4	18.86	0.63	3.2	9.43
5	B	hot	5	12.41	0.64	5.1	10.56
6	D	hot	8	10.83	0.57	5.2	9.03
7	D	cool	7	12.11	0.58	4.8	9.23
8	E	hot	11	8.83	0.49	5.6	10.16
9	E	cool	9	11.69	0.47	4.1	9.71
10	C	cool	6	11.02	0.53	4.9	8.52

\* No. 4 on special milk and bread diet = 26.6 grms. N.

As in all these experiments, with the exception of No. 4, the diets contained creatine, the quantities of creatinine excreted are probably slightly higher than that which represents tissue metabolism alone.

The figures, therefore, so far as they go, supply no evidence of a greater creatinine output in a tropical climate, nor do they show any marked seasonal variation.

#### URIC ACID

The average quantity of uric acid varied from 0.42 to 0.56 grams per day, which is quite a normal figure for a mixed diet.

### PURIN BASES

The considerable variations observed in the daily excretion of the purin bases are to be attributed to the tea and coffee of a mixed diet, since the greater part of these substances in the urine arise from this source. This is confirmed by the fact that with A on mixed diet (protocol III) these substances varied from 58 mgrms. to 92 mgrms. per day, whilst on a bread and milk diet (protocol IV, the quantity was much less and more constant, and varied during the next three days only from 21 to 28 mgrms.

### PHOSPHORIC ACID

The phosphoric acid varied very considerably, the average on mixed diet being between 1.56 and 2.61 grams per day. The nitrogen to  $P_2O_5$  ratio usually accepted for male Europeans is 5 or 6 : 1; in every case but one the average ratio found lay between 5.5 and 5.9, the exception showed a ratio of 7.1.

### CHLORIDES

The quantity of chlorides excreted was too variable in amount to allow of any conclusions.

### SULPHUR

The total sulphur when compared with the nitrogen gave ratios N : S of 14 to 15, this is somewhat higher than the average given in the text-books on Physiology, viz., 12.75, but Cathcart and Green (1913) during a number of determinations of this ratio, when different proteins were superimposed on a fixed diet, found in some cases numbers as high as this.

The quantities of neutral sulphur excreted were rather high, representing on a full protein diet from 11.8 to 20.8 % of the total sulphur, and averaging from 0.250 grams to 0.455 grams per day as  $SO_3$ . The average quantity on a full diet is, according to Folin, about 0.25 grms. per day, or about 5 % of the total sulphur. On a low protein diet he found no lessening in the amount excreted, the quantity then representing 25 % of the total sulphur. The inorganic sulphates and the ethereal sulphates were quite normal in amount.

### CONCLUSION

The number of subjects upon which the experiments were carried out is, of course, too few to draw any definite conclusions, but as far as they go they exhibit no marked variations from the averages obtained in temperate climates. In every case the neutral sulphur was high, but whether this is due to an increased tissue metabolism, or not, only further experiment will show.

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## PROTOCOLS

## I

SUBJECT A.—33 years; weight = 66.8 kilos; four years in Tropics

Date 1913	Volume cc.s.	Specific gravity	Total nitrogen grms.	CREATININE		
				grms.	As nitrogen grms.	per cent. of total nitrogen
Mar. 19 ...	1,487	1,015	14.20	1.65	0.61	4.32
„ 20 ...	1,425	1,013	10.25	1.47	0.55	5.32
„ 21 ...	1,030	1,028	15.31	1.51	0.56	3.67
„ 22 ...	775	1,027	9.17	1.59	0.59	6.44
„ 23 ...	690	1,026	9.52	1.55	0.58	6.05
„ 24 ...	875	1,025	12.07	1.56	0.58	4.80
„ 25 ...	745	1,026	9.61	1.89	0.70	7.31
„ 26 ...	880	1,027	12.12	1.99	0.74	6.10
„ 27 ...	970	1,022	12.93	1.52	0.57	4.37
Averages ...	986	1,023	11.69	1.64	0.61	5.38

## II

Date 1913	Volume cc.s.	Specific gravity	Total nitrogen grms.	CREATININE		
				grms.	As nitrogen grms.	per cent. of total nitrogen
Dec. 16 ...	730	1,029	12.43	2.24	0.83	6.70
„ 17 ...	675	1,028	11.31	1.93	0.72	6.34
„ 18 ...	895	1,024	12.38	2.00	0.74	6.00
„ 19 ...	755	1,027	9.60	1.67	0.62	6.46
„ 20 ...	875	1,026	12.04	1.92	0.71	5.92
„ 21 ...	820	1,028	11.37	2.02	0.75	6.60
„ 22 ...	720	1,030	11.26	1.78	0.66	5.86
„ 23 ...	705	1,028	12.24	1.73	0.64	5.25
Averages ...	772	1,027.5	11.58	1.91	0.71	6.14

III  
A.—Mixed Diet

Date	Volume ccs.	Gravity	P <sub>2</sub> O <sub>5</sub>	NaCl	Total N.	Urea	Ammonia	Creatinine	Uric acid	Purin bases mgms. N.	Sulphur as SO <sub>3</sub>			Ratio N. : S.	Ratio N. : P <sub>2</sub> O <sub>5</sub>
											Total S.	Inorganic sulphates	Ethereal sulphates		
12.2.14	1,100	1.022	2.00	10.85	12.74	24.67	0.51	1.74	0.44	58	2.271	1.661	0.194	14.0	6.4
13.2.14	1,105	1.022	2.41	13.47	12.82	24.41	0.53	1.82	0.44	73	2.227	1.713	0.186	14.4	5.3
14.2.14	1,045	1.024	2.66	12.03	14.59	26.29	0.62	1.67	0.55	81	2.491	1.886	0.178	14.6	5.9
15.2.14	930	1.028	2.90	9.60	12.75	23.04	0.62	1.54	0.39	64	2.688	2.104	0.142	11.8	4.3
16.2.14	1,425	1.020	3.03	10.90	17.28	33.40	0.91	1.72	0.49	62	3.159	2.182	0.294	13.7	5.7
17.2.14	1,000	1.026	3.05	8.50	15.69	29.43	0.68	1.67	0.38	92	2.692	2.013	0.175	14.6	5.1
18.2.14	885	1.027	2.23	7.42	14.47	27.61	0.57	1.58	0.41	59	2.352	1.767	0.203	15.0	6.5
Averages ...	1,070	1.024	2.61	10.38	14.33	26.98	0.63	1.68	0.44	70	2.554	1.904	0.196	14.0	5.5

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Date	Percentage of total N. excreted as					Percentage of total S.			
	Urea	Ammonia	Creatinine	Uric acid	Purin bases	Rest	Inorganic sulphates	Ethereal sulphates	Neutral S.
12.2.14	90.3	3.3	5.1	1.2	0.5	0	73.14	8.54	18.32
13.2.14	88.8	3.4	5.3	1.2	0.6	0.7	76.92	8.32	14.72
14.2.14	84.1	3.5	4.2	1.2	0.6	5.6	75.71	7.15	17.14
15.2.14	84.3	4.0	4.5	1.0	0.5	5.7	78.27	5.28	16.44
16.2.14	90.2	4.3	3.7	0.9	0.4	0.5	69.07	9.31	21.62
17.2.14	87.5	3.6	3.9	0.8	0.9	3.8	74.77	6.50	18.72
18.2.14	88.9	3.2	4.1	1.0	0.4	2.4	75.13	8.63	16.24
Averages	87.9	3.6	4.4	1.0	0.5	2.7	76.14	7.68	17.60

IV  
A.—Bread and Milk Diet

Date	Volume cca.	Gravity	P <sub>2</sub> O <sub>5</sub>	NaCl	Total N.	Urea	Ammonia	Creatinine	Uric acid	Purin bases mgms. N.	Sulphur as SO <sub>3</sub>			Ratio N. : S.	Ratio N. : P <sub>2</sub> O <sub>5</sub>
											Total S.	Inorganic sulphates	Ethereal sulphates		
4.3.14	1,015	1,024	2.47	8.70	17.01	33.05	0.70	1.75	0.47	56	3.283	2.667	0.201	12.9	6.9
5.3.14	1,200	1,023	3.22	13.19	18.8	36.61	0.76	1.67	0.43	28	3.165	2.694	0.120	14.9	5.9
6.3.14	1,165	1,023	3.48	13.14	19.24	36.57	0.73	1.73	0.49	21	3.276	2.786	0.111	14.7	5.5
7.3.14	1,152	1,023	3.69	12.99	20.32	37.90	0.78	1.71	0.46	23	3.315	2.842	0.107	15.3	5.5
Averages ...	1,133	1,023	3.28	12.00	18.86	36.03	0.74	1.71	0.46	32	3.260	2.747	0.135	14.4	5.9

103

Date	Percentage of total N. as					Percentage of total S.		
	Urea	Ammonia	Creatinine	Uric acid	Purin bases	Rest	Inorganic	Neutral
4.3.14	87.6	3.0	3.3	0.9	0.4	4.8	81.24	12.64
5.3.14	90.5	3.3	3.2	1.0	0.1	1.9	85.12	11.09
6.3.14	89.1	3.1	3.3	0.8	0.1	3.7	85.04	11.57
7.3.14	87.1	3.1	3.1	0.8	0.1	5.8	85.73	11.04
Averages	88.7	3.1	3.2	0.9	0.2	4.0	84.28	11.58

## V

Subject B.—25 years; weight = 60.6 kilos.; 4 years in Tropics; mixed diet.

Date	Volume ccs.	Gravity	P <sub>2</sub> O <sub>5</sub>	NaCl.	Total N.	Urea	Ammonia	Creatinine	Uric acid	Purin bases mgms. N.	Sulphur as SO <sub>3</sub>			Ratio N. : S.	Ratio N. : P <sub>2</sub> O <sub>5</sub>
											Total S.	Inorganic sulphates	Ethereal sulphates		
3.4.14	980	1.028	2.00	13.63	13.21	22.78	0.72	1.80	0.61	79	2.371	1.883	0.179	14.3	6.6
4.4.14	950	1.027	1.67	13.81	12.35	22.16	0.53	1.67	0.55	45	1.942	1.457	0.175	15.9	7.4
5.4.14	825	1.026	1.65	10.84	11.60	19.99	0.59	1.68	0.50	69	2.218	1.668	0.199	13.1	7.0
6.4.14	932	1.027	1.72	12.76	12.48	21.92	0.62	1.72	0.57	72	2.178	1.701	0.182	14.3	7.3
Averages...	922	1.027	1.76	12.76	12.41	21.71	0.61	1.72	0.56	64	2.177	1.677	0.184	14.4	7.1

104

Date	Percentage of total N. as					Percentage of total S. as			
	Urea	Ammonia	Creatinine	Uric acid	Purin bases	Rest	Inorganic	Ethereal	Neutral
3.4.14	80.5	4.5	5.1	1.5	0.6	7.8	79.42	7.55	13.03
4.4.14	83.7	3.6	5.0	1.5	0.4	5.8	75.03	9.01	15.96
5.4.14	80.4	4.2	5.3	1.4	0.6	8.1	75.20	8.97	15.82
6.4.14	81.3	4.1	5.1	1.5	0.6	7.4	78.13	8.36	13.51
Averages	81.5	4.1	5.1	1.5	0.5	7.3	76.94	8.47	14.58



## VI

SUBJECT C.—27 years; weight = 62.2 kilos; 2½ years in Tropics.

Date 1913	Volume ccs.	Specific gravity	Total nitrogen grms.	CREATININE		
				Grms.	As nitrogen	Percentage of Total N.
July 30 ...	1,540	1,011	10.86	1.44	0.53	4.94
" 31 ...	1,040	1,020	13.31	1.65	0.61	4.61
Aug. 1 ...	1,170	1,014	10.37	1.40	0.52	5.04
" 2 ...	1,480	1,014	11.31	1.45	0.54	4.76
" 3 ...	1,100	1,017	10.00	1.47	0.55	5.48
" 4 ...	1,255	1,015	10.84	1.46	0.54	5.02
" 5 ...	1,430	1,014	10.25	1.44	0.53	5.22
" 6 ...	1,625	1,010	10.42	1.30	0.48	4.65
" 7 ...	1,665	1,011	11.87	1.36	0.50	4.27
Averages ...	1,367	1,014	11.02	1.44	0.53	4.89

## VII

SUBJECT D.—30 years; weight = 62.8 kilos; 1-1½ years in Tropics.

Date, 1913	Volume ccs.	Specific gravity	Total nitrogen grms.	CREATININE		
				Grms.	As nitrogen grms.	percentage of Total N.
July 30 ...	2,340	1,011	13.72	1.80	0.67	4.87
" 31 ...	970	1,024	12.15	1.40	0.52	4.27
Aug. 1 ...	1,540	1,018	12.28	1.66	0.62	5.02
" 2 ...	1,730	1,012	12.26	1.85	0.69	5.60
" 3 ...	970	1,024	11.85	1.48	0.55	4.65
" 4 ...	1,190	1,019	13.86	1.59	0.59	4.42
" 5 ...	1,310	1,015	12.10	1.45	0.54	4.45
" 6 ...	1,430	1,015	11.51	1.53	0.57	4.95
" 7 ...	1,950	1,012	9.71	1.29	0.48	4.92
" 8 ...	940	1,020	11.62	1.50	0.56	4.79
Averages ...	1,437	1,017	12.11	1.55	0.58	4.79

VIII  
D.—Mixed Diet : 63.1 kilos

Date	Volume ccs.	Gravity	P <sub>2</sub> O <sub>5</sub>	NaCl.	Total N.	Urea	Ammonia	Creatinine	Uric acid	Purin bases mgms. N.	Sulphur as SO <sub>3</sub>				Ratio N. : S.	Ratio N. P <sub>2</sub> O <sub>5</sub>
											Total S.	Inorganic sulphates	Ethereal sulphates	Neutral S.		
6.4.14	890	1.025	1.68	11.68	10.86	18.79	0.55	1.54	0.37	103	2.082	1.575	0.088	0.419	13.0	6.5
7.4.14	710	1.026	2.14	7.05	11.46	20.79	0.55	1.51	0.47	77	1.962	1.412	0.156	0.394	14.6	5.4
8.4.14	790	1.028	2.06	6.84	11.00	19.72	0.56	1.67	0.47	78	2.110	1.539	0.149	0.422	13.7	5.3
9.4.14	470	1.028	1.47	3.88	8.78	15.19	0.45	1.26	0.34	28	1.375	0.928	0.096	0.351	15.8	6.0
10.4.14	832	1.025	2.03	6.98	12.66	22.95	0.56	1.74	0.51	157	2.086	1.440	0.196	0.450	14.5	6.2
11.4.14	790	1.027	1.93	8.25	10.30	17.98	0.45	1.47	0.37	118	1.754	1.270	0.170	0.308	14.7	5.3
12.4.14	995	1.020	2.03	6.98	10.74	19.43	0.41	1.54	0.45	82	1.506	0.995	0.200	0.311	17.8	5.3
Averages ...	782	1.025	1.96	7.38	10.83	19.26	0.50	1.53	0.42	92	1.836	1.308	0.151	0.379	14.9	5.7

Date	Percentage of Total N. as					Percentage of Total S. as			
	Urea	Ammonia	Creatinine	Uric acid	Purin bases	Rest	Inorganic	Ethereal	Neutral
6.4.14	80.8	4.1	5.2	1.1	0.9	7.9	75.65	4.23	20.12
7.4.14	84.6	3.9	4.9	1.4	0.7	4.5	71.97	7.95	20.08
8.4.14	83.6	4.2	5.6	1.4	0.7	4.5	72.94	7.06	20.00
9.4.14	80.8	4.2	5.3	1.2	0.3	8.2	67.49	6.98	25.58
10.4.14	84.6	3.6	5.1	1.3	1.2	4.2	69.03	9.40	21.57
11.4.14	81.4	3.6	5.3	1.2	1.2	7.3	72.40	10.03	17.56
12.4.14	84.4	3.2	5.3	1.4	0.8	4.9	66.07	13.28	20.65
Averages	82.9	3.7	5.2	1.3	0.8	6.3	69.36	8.42	20.80

SUBJECT E.—36 years; 48.4 kilos; 1 to 1½ years in Tropics

Date, 1913	Volume ccs.	Specific gravity	Total nitrogen grms.	CREATININE		
				Grms.	As nitrogen grms.	Percentage of Total N.
July 6 ...	790	1.022	9.04	1.28	0.48	5.3
" 7 ...	915	1.023	10.47	1.32	0.49	4.7
" 8 ...	970	1.022	11.79	1.28	0.48	3.9
" 9 ...	1,185	1.016	10.19	1.22	0.45	4.5
" 10 ...	1,118	1.018	11.36	1.22	0.45	4.0
" 11 ...	1,510	1.016	11.50	1.34	0.50	4.3
" 12 ...	895	1.024	12.60	1.28	0.48	3.8
" 13 ...	850	1.026	13.08	1.20	0.45	3.4
" 14 ...	1,370	1.015	14.30	1.21	0.45	3.1
" 15 ...	770	1.026	12.54	1.31	0.49	3.9
Averages ...	1,037	1.021	11.69	1.27	0.47	4.1

## X

E. Weight = 48.2 kilos.

Date, 1914	Volume ccs.	Specific gravity	Total nitrogen grms.
June 25 ... ..	1,540	1.011	9.97
" 26 ... ..	1,150	1.013	10.86
" 27 ... ..	1,460	1.012	10.77
" 28 ... ..	1,340	1.013	10.50
Averages ...	1,372	1.012	10.52

XI  
E.—Mixed Diet

Date	Volume cca.	Gravity	P <sub>2</sub> O <sub>5</sub>	NaCl.	Total N.	Urea	Ammonia	Creatinine	Uric acid	Purin bases mgms. N.	Sulphur as SO <sub>3</sub>				Ratio N. : S.	Ratio N. : P <sub>2</sub> O <sub>5</sub>
											Total S.	Inorganic sulphates	Ethereal sulphates	Neutral S.		
18.3.14	540	1,024	1.63	3.80	8.04	13.62	0.47	1.38	0.53	67	1.293	0.935	0.109	0.249	15.5	4.9
19.3.14	555	1,024	1.69	3.81	8.88	14.55	0.36	1.32	0.52	67	1.447	1.105	0.120	0.222	15.3	5.2
20.3.14	505	1,025	1.61	3.92	9.12	14.98	0.57	1.32	0.50	56	1.441	1.209	0.078	0.154	15.8	5.7
21.3.14	600	1,024	1.61	5.84	8.74	14.19	0.49	1.29	0.45	60	1.396	1.014	0.120	0.262	15.7	5.4
22.3.14	673	1,023	1.49	5.70	8.18	13.28	0.49	1.26	0.47	95	1.581	1.108	0.199	0.274	12.9	5.5
23.3.14	804	1,020	1.45	5.40	9.46	16.07	0.64	1.39	0.52	76	1.707	1.359	0.135	0.213	13.8	6.5
24.3.14	640	1,024	1.44	6.52	9.43	16.85	0.57	1.27	0.39	73	1.496	0.960	0.157	0.379	15.8	6.5
Averages ...	621	1,023	1.56	5.00	8.83	14.79	0.51	1.32	0.48	71	1.480	1.099	0.131	0.250	15.0	5.7

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Date	Percentage of total N. as				Percentage of total S. as			
	Urea	Ammonia	Creatinine	Uric acid	Purin bases	Rest	Inorganic	Ethereal
18.3.14	79.1	4.8	6.3	2.2	0.8	6.8	72.31	8.43
19.3.14	77.4	3.4	5.6	1.9	0.8	10.9	76.36	8.29
20.3.14	76.6	5.1	5.4	1.8	0.6	10.5	83.90	5.41
21.3.14	77.5	4.6	5.5	1.7	0.7	10.0	72.63	8.60
22.3.14	75.8	4.9	5.7	1.9	1.2	10.5	70.08	12.59
23.3.14	79.3	5.6	5.5	1.8	0.8	7.0	79.61	7.91
24.3.14	83.3	5.0	5.0	1.4	0.8	4.5	64.17	10.49

# THE SPECIES OF *PARAGONIMUS* AND THEIR DIFFERENTIATION\*

BY

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*(Received for publication 23 September, 1914)*

## PLATES VII-XI

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### INTRODUCTION

In 1895 the senior author published the first account of the occurrence of a mammalian lung fluke on the North American Continent. On various occasions since then he has contributed to the knowledge of these forms. In 1910 the junior author found some of these parasites in a host which was indisputably a native of Wisconsin, and became much interested in the problems connected with the discovery, so that when in the following year he engaged in graduate study at the University of Illinois this topic was naturally selected for his work. The present paper represents the results of that work and of the contemporaneous and subsequent

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\* Contributions from the Zoological Laboratory of the University of Illinois, No. 34.

studies of the senior author. Unfortunately, even after very considerable delay, it has proved impossible to obtain all of the material from distant regions essential for comparison and determination of all the species which are involved in the literature of the subject. But we feel clear that the results, even if imperfect still in certain details, should be laid before scientific workers in this field without further delay that they may be tested by those more favourably situated to secure material in quantities.

### HISTORICAL SUMMARY

The earliest record of a lung fluke in mammals was published by Kerbert in 1878. During an autopsy held in the Zoological Gardens at Amsterdam, Holland, in September, 1877, parasites were found in the lungs of a tiger, and were sent by the Director, Westerman, to Kerbert, who determined them to be undescribed trematodes and named the species *Distoma westermanii*. Three years later Kerbert received specimens, through Bolau, from the lungs of a tiger that had died in the Zoological Gardens at Hamburg, Germany. Upon this material, which proved to be identical with the former, he completed an extensive morphological study (Kerbert, 1881) of the species.

The first information concerning the occurrence of such a parasite in man came from Manson, who in a letter to Cobbold, published with comments by the latter, described (Manson, 1880) the discovery of distome eggs in the sputum of a Chinaman suffering from hemoptysis. At the same time he mentioned a fluke found in another patient, a Portuguese, by Dr. Ringer. The specimen was sent to Cobbold, who in the note just cited named it *Distoma ringeri*. Although the description is scanty, the records leave no doubt as to the identity of the species. It should be noted that this is the first published record of this human parasite, antedating by nearly two months that of Baelz, to which priority is often accorded.

The latter record came thus almost simultaneously, but from Japan. As early as 1878 Baelz had noted these conditions, similar to tuberculosis and regularly attributed to it, which he regarded as resulting from some parasitic infection. He sent the sputum to

Leuckart for examination and described the bodies in it as psorosperms, but before receiving Leuckart's diagnosis, came to the conclusion that they were in reality eggs of some fluke. This opinion was first published by Manson (1882), who stated that after having examined some of Baelz' gregarine material he had found bodies identical with the ova of *Distoma ringeri*. In the following year Baelz (1883), confirmed in his view of the nature of the material by the report of Leuckart, published a description of the worm, which he re-named *Distoma pulmonale*. According to Stiles and Hassall (1900, p. 567), certain Japanese investigators in the interval had named the species *Distoma pulmonis*. Both this name and that of Baelz are ante-dated by the name of Cobbold, which is apparently the first designation used for the human parasite. There is no need to review here the important clinical and pathological data submitted by Manson, Baelz, and later writers. The wide and abundant distribution of the parasite in Japan, Formosa and Korea, its occurrence among all ages and classes of society, and the high ratio of the infected in certain districts are well-known and firmly-established facts. The Asiatic lung distome is one of the most important human parasites.

The next important step in the history of this form was taken by Leuckart, who in conjunction with his student Nakahama made a careful study of specimens of the fluke which Baelz had sent him and of specimens of *Distoma westermanii* from Kerbert. Although he noted points of disagreement, these were interpreted as unimportant minor differences, and the specific identity of the two forms was definitely asserted. We do not find that this identity has been questioned since then, except once (Ward, 1908). Stiles and Hassall (1900, p. 561) voice the general opinion in stating that the human parasite 'though originally supposed to represent a new species is now generally admitted to be identical with Kerbert's form from the tiger.'

Regarding the genus, however, several investigators commented independently on the impossibility of retaining this form in the old group *Distoma*; and finally in 1899 Braun made it the representative of a new genus to which he gave the name *Paragonimus*. The first careful and extended description of the genus came from Looss (1899), who unaware of Braun's publication gave the form

another name that, because of its slightly later appearance, must be relegated to synonymy. However, the description given by Looss (1899) still ranks as the most accurate and complete available. Even at that time Looss recognized only a single species, to which he gave the name *Paragonimus westermanii*.\*

Except for its sporadic extra-limital occurrence in hosts that were known to have come from the regions where the parasites are endemic, the species was not recorded from the western continent until one of us (Ward, 1894) published a description of a similar form obtained from a cat in Michigan. This paper called attention to the differences between these worms and the description given by Kerbert and Leuckart for *Distoma westermanii*, but assigned them tentatively to the same species. It also noted the possibility that the host had been brought as a pet from the East, and that hence the parasite was not endemic. Grave doubt was thrown on this view through the discovery by Kellicott of worms in the lung of a dog from Ohio, which were sent to Ward and pronounced by him identical with those he reported from the cat.

All possibility that the parasite had been found only in hosts introduced from abroad disappeared when Stiles and Hassall (1900) recorded the abundant discovery of a similar parasite from hogs slaughtered at Cincinnati, Ohio. Their paper gives not only a very careful account of these specimens but a detailed comparison with other records and the best critical summary of the literature on these lung flukes which has been published to date. While recognizing the possibility that their worms 'may represent a distinct variety at least,' Stiles and Hassall 'feel compelled to continue for the present to look upon the American form as identical with the Asiatic.'

Since then the lung fluke has been reported in numerous cases from North America. In the human host it was first diagnosed by Mackenzie, in 1904, from the lung of a Japanese fisherman on the Columbia river. Then Fehleisen and Cooper (1910) reported a case in a Japanese worker in California fruit orchards who had come to the country some six years before. Microscopic examination of the sputum showed in every field two or three eggs of the distome. No details of size or structure are given, but the case was

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\* Looss wrote *P. westermanni* which is evidently a typographical error.



of long standing and had been diagnosed in Japan as lung fluke. Thus all the evidence goes to show that it was not contracted in the United States. In the same year Null (1910) recorded its occurrence in a Korean at Seattle, Washington.

From other hosts there are several cases on record. Null stated casually that it occurs in dogs and cats from the Oriental quarters of San Francisco, but gave no further data regarding the parasites. Nickerson (1911) recorded the occurrence of *P. kellicotti* in the lung of a cat from the grounds of the University of Minnesota at Minneapolis. Only three specimens were found.

The junior author of this paper (Hirsch) observed several cases of marked significance in Wisconsin. As early as 1907 he noticed a peculiar cough in a pet cat, which became more seriously affected and was chloroformed a year or more later. These worms were found in the lungs. A kitten born to the first cat was affected in the same way and found to be infected with the lung fluke when only a little more than a year old. In addition to these cases, of which the full case history, written by Hirsch, was published by Hanson (1911, p. 112), the former has also found the lung fluke in two other cases hitherto unpublished. These cases are another kitten of the same litter and a fourth cat, unrelated and living some miles distant from the first three. It is positively known that the first three were born in that locality and had never been away from it, hence the endemicity of the parasite can only be questioned on the ground that possibly the means of infection—a fish carrying the encysted young distome for instance—was brought in from a distance. There is no probability that the two young cats were infected directly from the mother cat, but in all likelihood through the consumption of the same food. It seems improbable that all three could have been infected at the same date, and certainly the fourth cat did not acquire its parasites from the same food. These cases are the first in which the presence of the lung fluke was diagnosed in the living animal and the diagnosis confirmed by the demonstration of the ova in the sputum. They are also the first on this continent in which the place of birth and infection was positively determined for the host.

The range of the human lung fluke was further extended by the work of Musgrave (1907). He studied seventeen cases with eight

autopsies in the Philippine Islands, and gave a careful morphological description of the parasite. His data will be utilised later in our paper.

One of the seventeen hosts was a Chinaman, two were Japanese, and the other fourteen were native Filipinos, so that the species is undoubtedly endemic in the Islands. Various later papers contain casual references to the occurrence of this species in the Islands, but do not give data of value for our purposes. Garrison and Leynes (1909) studied the development of the ova of *Paragonimus* in the Philippines. They do not give a precise description of the ova, but apparently these were obtained from cases among those recorded by Musgrave (1907) which have already been considered. The work of Garrison and Leynes, which deals with the experimental development of *Paragonimus* ova under varying conditions of temperature, light, salinity and desiccation, has bearings of great importance on the dissemination of the disease and the infection of man, but does not throw any light on the problem of species and their differentiation.

In a paper published several years ago, the senior author (Ward, 1903) commented on the great discrepancies between the measurements recorded by various investigators for the eggs of *Paragonimus westermanii*, and gave an outline sketch representing these differences in a graphic manner. He reached the conclusion that all records could not be accepted as correct unless more than one species was concerned. The measurements given by Yamagiva, which were made from ova in sections of the brain and lungs of man, have since been shown to belong, in all probability, as Ward stated later, to the Japanese blood fluke, *Schistosoma japonicum*, and are consequently eliminated from the present discussion. In a later paper (Ward, 1908) these records were discussed at greater length in the light of further evidence, and the conclusion was reached that the American form originally identified as *Paragonimus westermanii* is undoubtedly a distinct though closely-related species, and to it Ward gave the name of *Paragonimus kellicotti*. He also indicated the probable specific independence of the Japanese form on which further work was then being done. This form, which will be fully discussed in the descriptive section of this paper, must bear the name of *Paragonimus ringeri* (Cobbold, 1880).

## MATERIAL

The junior author made a detailed and careful study of *Paragonimus kellicotti* on new material obtained from the pig at the Cincinnati (Ohio) abattoirs. The specimens were taken alive from the lungs, and after shaking in normal salt solution according to the method of Looss, were preserved with great care. The technique involved nothing unusual, and gave good results throughout. Total preparations and serial sections of the specimens originally obtained by Ward from the cat in Michigan, by Kellicott from the dog in Ohio, and by Hirsch from the cat in Wisconsin, were compared item by item with this new American material.

Several specimens of the Japanese form from man were also available for comparative study through the courtesy of Dr. S. Uchida, of Tokyo, and eggs from the Seattle case were kindly sent us by Dr. Null. This form has been described in detail several times, notably by Katsurada (1900) and Kubo (1912). Despite this fact, the structural features are not even yet well known, and in most respects these descriptions are couched in such general language that we cannot determine from the paper more than generic features regarding certain organs. The last paper especially (Kubo, 1912) falls short of what might be wished. In spite of a rich supply of fresh material he gives very little more information on the structure than the work of Katsurada a dozen years earlier. The figures are distinctly unsatisfactory, being in some cases vague and almost illegible, and in others highly diagrammatic. They are certainly inferior to those given by Katsurada.

Our work was supplemented by comparison with three co-types of *Distoma westermanii* kindly placed at our disposal by Professor Kerbert and now in the Ward collection. While this supply was adequate for the determination of the most important features in the anatomy, as will appear in the following pages, it did not suffice for a complete study of the structure, and a detailed comparison of this form with those from man in Japan, and from cat, dog, and hog in North America, must be left to some future student who has at his command a larger supply of material.

Despite persistent effort, it has been impossible to secure for study and comparison any material from the Philippine Islands,

so that the status of that form could not be tested by the method worked out on the other material. Although only a single paper has been written on the anatomy of this form, that one is so carefully worked out that it furnished very definite material for comparison with the work on other species. In spite of this, there are several points on which we would fain have had precise information concerning the structure of some organ that was not treated *in extenso* in the text of that paper.

### STRUCTURE OF *PARAGONIMUS*

#### *General Form*

*Paragonimus kellicotti* has a somewhat elongated form, elliptical in dorsal aspect. The dorsum is strongly arched, the highest point being somewhat anterior to the middle portion of the body, while the ventral surface is slightly flattened. The anterior end rounds off gradually, but the posterior extremity is attenuated, and sharply curved. The range in size is given in comparison with *Paragonimus ringeri* and *Paragonimus westermanii* in the following table:—

Species	Host	Length in mm.			Width in mm.			Thickness in mm.			Authority
		Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	
<i>P. kellicotti</i>	... Hog	11.0	8.5	9.8	3.5	3.0	3.1	3.2	2.2	2.6	Hirsch
<i>P. kellicotti</i>	... Cat	15.5	11.2	13.6	7.7	4.8	—	—	—	—	Ward
<i>P. kellicotti</i>	... Hog	14.0	3.0	—	4.0	2.0	—	—	—	—	Stiles
<i>P. kellicotti</i>	... Dog	20.0	15.0	—	—	—	—	—	—	—	Kellicott
<i>P. westermanii</i>	... Tiger	9.0	7.0	—	6.0	4.0	—	4.0	2.0	—	Kerbert
<i>P. ringeri</i>	... Man	10.0	8.0	—	6.0	5.0	—	—	—	—	Leuckart
<i>P. ringeri</i>	... Man	13.0	7.1	9.6	7.5	5.0	5.0	4.0	3.5	3.7	Katsurada
<i>P. ringeri</i>	... Man	17	7.5	10.1	6	4.5	5.8	5.5	3.5	5	Kubo

From these figures it may be noted that there is considerable variation in the size of the different individuals. Taken as a composite picture, however, a description of the American form emphasizes its much elongated, relatively slender structure, which is in contrast with *Paragonimus ringeri* and *Paragonimus westermanii*, both of which are oval-shaped, thicker, and broader parasites.

The most recent and extensive study of fresh material for *Paragonimus ringeri* has been made by Kubo (1912). He has listed measurements of thirty-six specimens from the lungs of the dog in Japan and of eleven specimens from the human lung in the same region. There seems to be no contrasted difference between specimens from the two hosts. The entire series averages in length 10.08 mm.,\* in width 5.8 mm., and in thickness 5 mm. The smallest he found measured 3 mm. in length and the largest 12 mm. Worms with a length of 5 mm. and over are sexually mature, having some eggs in the uterus. He has not included these extremely small and presumably young specimens in his averages, so that the latter represent fairly well full-grown adults. Yet even with that, the omission of five conspicuously small specimens listed in his table will raise the size averages appreciably.

On the other hand, when one examines a group of these parasites with the eye it is not difficult to see a general difference between the species in type of form. Yet at the same time it is clear that neither the size nor the form gives a safe basis for distinguishing the species. There is no doubt that some of the conflicting views in regard to size are due to the measurement of specimens at different ages and stages of growth.

At the anterior extremity is found the oral sucker directed towards the ventral surface at an angle of about 45°, while the acetabulum lies on the same surface in the median line slightly anterior to the centre of the body. In ten specimens these suckers average respectively 0.75 mm. and 0.83 mm. in diameter. The two suckers of *Paragonimus westermanii* are of about equal size, 0.78 mm. in diameter, according to Leuckart, while those of *Paragonimus ringeri* are unequal, and smaller, the oral sucker

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\* Not 10.8 mm. as given by Kubo in the text.

being 0.53 mm. in diameter and the acetabulum 0.6 mm., or at most 0.75 mm. according to Leuckart.

Kubo says that the oral sucker of *Paragonimus ringeri* in a medium-sized worm (10 mm. long) measures 0.75 mm. in diameter, and that the ventral sucker in a similar specimen reaches 0.8 mm., being thus slightly larger than the oral sucker. These measurements differ from those of Leuckart and agree closely with those we report for *Paragonimus kellicotti*, but here again the character varies so much with the size and age of the specimen measured that no dependence can be placed upon it in determining the species.

The genital pore in *Paragonimus kellicotti* lies just behind the acetabulum, medial or a little to the right or to the left. The position of the genital pore as given by Stiles for other species is the same. The cuticula covering the entire body is relatively thick, and armed with spines (Pl. X, fig. 18). The thickness of this structure, however, is not uniform over the entire body. Around the suckers it may be 0.005 mm. thick, while over the posterior extremity it may be 0.048 mm. There is often also a distinct difference in the appearance of this layer. Sometimes it seems perfectly homogeneous, but in other places it appears as if made up of two distinct layers, an outer more dense and less refractive, and an inner vertically striated, rather clear, or highly refractive with numerous coarse granules. The cuticula shows no cellular structure, but is sharply marked from the underlying muscles by a distinct basement membrane.

### *Cuticular Spines*

Since a preliminary study of the spines revealed characteristics that appeared to be of great value for the differentiation of species, these structures were subjected to a most precise examination, with results that we believe justify the labour. It should be recalled that Kerbert, Leuckart, and Stiles differ in their statements concerning the distribution of these structures and the place where the largest are to be found, but so far as we can ascertain no one has noted differences in the form of individual spines or in their grouping. An examination of the three worms with reference to

these specific features gave pictures interesting both for their resemblances and for their striking differences. The conditions in the parasite from the lung of the pig are given first.

The spines of *Paragonimus kellicotti* lie in irregular circular rows over the body and are set firmly in the cuticula with their free ends directed posteriad. They extend entirely through the cuticula, and sometimes into the body musculature beneath (Pl. X, fig. 18). Structurally, the spines are thin chisel-shaped scales, usually several times as long as wide. The surface directed away from the body is slightly convex, while that directed towards the body is correspondingly concave. The free end is rather deeply serrated into a number of very sharp teeth. These certainly aid the parasite materially in effecting its movements, and in maintaining its position in the tissues of the host. The basal end of the spines is broader and thicker (0.005 to 0.010 mm.) than the tip, often appearing cleft so as to show in cross-section groups of closely aggregated oval or irregularly shaped chitinous bodies.

There is a distinct difference in the distribution and size of the spines (Pl. VIII, figs. 5-11) in various parts of the body. The suckers are entirely devoid of these structures, while the cuticula closely surrounding shows the transition stages between the non-spined and the spined condition. This holds true especially for the region surrounding the acetabulum. Here the body for a small distance around the sucker may be entirely free from spines. When they appear, they are few in number but very sharp and even decidedly hooked (fig. 10). Around the oral sucker the spines (fig. 9) are short with broad bases and sharp tips, a condition which is soon replaced by larger spines with broad serrated tips. The spines are set relatively more thickly over the anterior half of the body (fig. 5) than over the posterior (fig. 7). The cuticula on the dorsal surface just behind the oral sucker, and on the ventral surface between the suckers (fig. 8) and just behind the acetabulum, is especially well armed. The spines over the dorsal surface show little variation except in number and in length. On the ventral surface the greatest variation occurs around the suckers. This divergence, however, is not one from the characteristic type, but rather is a gradual diminution in size. The spines on this surface of the body are perhaps shorter and broader than over the dorsum.

The results of an extensive series of measurements of the spines in *Paragonimus kellicotti* have been brought together in the form of a table or summary. This shows the exact size of individual spines in various regions of the body, their distance apart, and their variation in those cases where marked differences occur (Table A).

TABLE A.—SPINE MEASUREMENTS OF *Paragonimus kellicotti*

	Distance apart	Length	Base	Tip ]
<b>VENTRAL SURFACE—</b>				
Near Oral Sucker ... ..	0.005—0.008 mm.	0.010 mm.	0.005 mm.	0.000 mm.
Between Suckers ... ..	0.013—0.026 mm.	0.046 mm.	0.013 mm.	0.013 mm.
Acetabular Region... ..	0.030 mm.—variable	0.010—0.020 mm.	0.001 mm.	0.000 mm.
Between Acetabulum and Anterior End	0.026 mm.	0.031 mm.	0.013 mm.	0.008 mm.
Between Acetabulum and Posterior End	0.013—0.026 mm.	0.039 mm.	0.023 mm.	0.021 mm.]
Posterior Extremity ... ..	0.026—0.047 mm.	0.036 mm.	0.015 mm.	0.008 mm.
<b>DORSAL SURFACE—</b>				
Anterior Extremity ... ..	0.008—0.012 mm.	0.031 mm.	0.010 mm.	0.008 mm.
Anterior One-Fourth ... ..	0.012—0.026 mm.	0.034 mm.	0.010 mm.	0.008 mm.
Middle of Body ... ..	0.015—0.026 mm.	0.044 mm.	0.010 mm.	0.008 mm.
Posterior One-Fourth ... ..	0.026—0.036 mm.	0.040 mm.	0.010 mm.	0.008 mm.
Posterior Extremity ... ..	0.012—0.026 mm.	0.036 mm.	0.010 mm.	0.008 mm.]

The spines of *Paragonimus kellicotti* are of one general type, chisel-shaped, with distinctly serrated or saw-toothed free extremities. There is, to be sure, a variation in their length, but in general the characteristic features of this type of spine are retained throughout. The study of these spines involved their examination over the entire body of the parasite; in no place was there any marked divergence in form, and nowhere did they occur other than singly. While the substance of the comparison was made upon material taken from the hog, further data were obtained by examining a piece of the cuticula from a parasite removed from the lungs of a cat. The shape of these spines, as well as their



arrangement, was the same (Pl. VIII, fig. 11), the only difference noted was that in extreme cases they were somewhat longer (0.078 mm.), and their free ends more deeply serrated. Such differences may easily be due to the age of the individual parasite. Our material was not extensive enough to enable us to test the question of a possible growth in the size of these spines during the life of the adult parasite. But we think all will grant that this extreme of variation in the cuticular spines is not great enough to justify regarding them as separate and distinct from the type found in the hog, much less to conceal their essential agreement with that type. Their radical dissimilarity with the other types will appear conspicuously when those types have been described.

The dimensions of the spines of *Paragonimus westermanii*, as given by Kerbert, have elicited considerable comment, for he reports that the largest spines were 0.018 mm. long by 0.002 mm. broad at the base, and the others were 0.010 mm. long. He describes the spines as lancet-shaped. Kerbert's figure shows the posterior end of the parasite and the surface for a short distance around the oral sucker entirely devoid of spines. Upon re-examining the co-type specimens of *Paragonimus westermanii* in the Ward Collection, these statements were found to need some emendation. The spines are indeed lancet-shaped, but are considerably longer than Kerbert's dimensions indicate. They are fully as long as those in the cuticula of *Paragonimus kellicotti*, being 0.047 to 0.049 mm. long by 0.005 to 0.010 mm. broad at the base. In addition, spines cover the entire body excepting the suckers, as in *Paragonimus kellicotti*. Leuckart gives the measurements of the longest spines of *Paragonimus ringeri* as 0.06 mm. with a base of 0.014 mm. broad. These dimensions approximate the size condition revealed in *Paragonimus kellicotti* by our investigations.

To judge from these data one would be forced to maintain that there was no clear difference between the spines in the three species, since all of the measurements fall well within the extremes we found in *Paragonimus kellicotti*. And yet when one takes into account the form of the individual spine and the arrangement of the spines on the surface of the body there are evident and distinctive differences of a striking character. The measurements

already given show that the spines of *Paragonimus westermanii* and those of *Paragonimus ringeri* do not differ in size much, if at all, from those of *Paragonimus kellicotti*. In the first two species, however, the spines are markedly lancet-shaped, while in the last-named they are, as already stated, broad and chisel-shaped. The condition in *Paragonimus ringeri* is different. Over certain parts of the body, especially on the ventral surface behind the acetabulum, the spines are short with broad bases and tips. Here they are set very closely together, forming at times almost continuous circular rows. Along the side of the body the spines are slender with sharp free ends.

While these differences in shape are very striking characteristics, the spine arrangement is even more distinctive. The spines in *Paragonimus westermanii* lie rather sparsely scattered in more or less incomplete circular rows (Pl. VIII, fig. 13). Their free ends extend very far beyond the cuticula, giving the parasite a thorny appearance. In *Paragonimus ringeri* (Pl. VIII, fig. 12) the general appearance is very different, since the spines are characteristically arranged in groups which are often massed together, and in certain parts of the body are close enough to form almost continuous circular rows. While the shape of the individual spines in *Paragonimus ringeri* is subject to some variation, in fact offering a transition series between *Paragonimus westermanii* and *Paragonimus kellicotti*, the group arrangement affords a striking and constant means of differentiation between the species.

In his description of the Philippine form, Musgrave (1907, p. 32) discusses the distribution of the spines at length. He says that on the ventral surface between the acetabulum and the oral sucker they are 'almost entirely absent,' and that when they occur in this region they are 'smaller than the others,' whereas they increase in size on the lateral borders and reach a maximum on the dorsum. This does not agree with conditions in the forms we have studied and have described in the foregoing, as is shown distinctly by a comparison of this statement with our figures. Musgrave speaks of these spines as 'scale-like,' a term difficult to compare with our findings, and he gives no illustration of these structures. From the evidence at hand we are unable to reach a final conclusion, but it favours the separation rather than the amalgama-

tion of these forms with any known species. The decision of this matter must await the study of material from the Philippine Islands, which as yet we have been unable to secure.

In regard to the Japanese form, Kubo gives no data that can be compared with our findings, but in one part of this description he says that in larger and older worms the spines lose their sharp points and become stumpy. This might suggest the interpretation of the conditions we have described as possibly changes with growth and use, dependent thus merely upon the age of the specimens. This view is hardly tenable, since our illustrations are taken from specimens of the same approximate size, and presumably of the same age, if all were a single species. Furthermore, this explanation does not touch the striking differences in the arrangement of the spines on the surface of the body.

The differences in form and arrangement of the cuticular spines in the species we have studied may be summed up in tabular form as follows:

	<i>P. westermani</i>	<i>P. ringeri</i>	<i>P. kellicotti</i>
Shape ... ..	Lancet-shaped Very slender	Chisel-shaped Moderately heavy	Chisel-shaped Heavy
Distribution ... ..	Sparsely, somewhat irregularly, singly	Circular rows, in groups	Circular rows, singly

### *The Alimentary System*

The opening leading from the oral sucker into the pharynx in *Paragonimus kellicotti* measures 0.050 to 0.075 mm. in diameter. It becomes somewhat larger toward the outer margin of the sucker, and in sectional view appears wedge-shaped. It is continuous proximally with a thin lamella that forms a small pocket, the pre-pharynx, which is located between the pharynx and the sucker and serves to unite the two. No such region is mentioned by other authors, and yet may be found in *Paragonimus westermanii* or *Paragonimus ringeri* on careful examination.

The entrance into the pharynx is provided with four lip-shaped

projecting folds which effectively close the canal during contraction. The two which lie laterally are larger than those bounding the tube dorsally and ventrally, thus making the entrance into the pharynx appear much like a vertical slit. The pharynx is spherical in shape, about 0.4375 mm. in diameter. Its walls are composed of heavy muscles, and the canal itself is limited to a narrow vertical slit. The inner lining of the pharynx consists of a thin layer of the cuticula, 0.01 to 0.02 mm. thick. Towards its union with the oesophagus the opening through the pharynx widens considerably.

The oesophagus is nearly circular in trans-section, and about 0.21 to 0.255 mm. long. The anterior part of the alimentary canal is directed towards the dorsal wall, but not very acutely. After the junction of the pharynx with the oesophagus this direction is continued, and becomes a little more abrupt. At first the oesophagus is about 0.08 mm. in diameter, but towards its branching this dimension decreases slightly and then increases again to 0.16 or even 0.175 mm. The thickness of the wall varies in this short distance from about 0.012 mm. near the pharynx to 0.05 mm. somewhat nearer the bifurcation. This difference necessarily causes the inner lumen to vary inversely. Such relations as have been described are subject to variations no doubt due to the contraction of these parts. Structurally the oesophagus (Pl. XI, fig. 23) is relatively simple, consisting of an inner homogeneous layer, often thrown up in folds, and an outer well-developed muscular layer. The latter is made up essentially of the inner circular fibres, while the outer longitudinal fibres surround the wall in relatively heavy parallel bands. This portion of the alimentary canal is enveloped by well-developed glandular cells whose ducts open into the oesophagus, in all probability furnishing a salivary or digestive secretion.

The two lateral branches of the oesophagus are 0.175 to 0.255 mm. long, and are sharply differentiated from the intestinal caeca by several distinct characters. The intestinal caeca immediately attain a diameter of 0.315 mm., whereas the oesophageal branches measure only 0.05 mm. in this dimension. More generally speaking, there is in this region a sudden increase in the diameter of the caeca to about four or five times that of the oesophageal branches. In addition, the intestinal epithelium is

characterized by tall columnar cells (Pl. X, fig. 17) which contrast strikingly with the lining of the branches of the oesophagus.

The further course of the caeca is readily followed on the reconstructions (Pl. IX, figs. 14, 15, 16) made from a typical specimen of *Paragonimus kellicotti* that was imbedded and sectioned without having been subject to pressure or other distortion. After the bifurcation of the oesophagus into the two lateral branches, these ascend rather rapidly toward the outer and upper margins of the body, and come to lie close under the vitellaria, but not widely separated from each other. This relation is apparent inasmuch as the body of *Paragonimus kellicotti* in a natural condition is not broad or flattened but attenuate and rounded. After its short transverse passage, each of the intestinal caeca passes backward toward the posterior extremity of the body, where they end blindly, one on each side, quite close together. The termination of each canal takes place at about the same level, although a slight variation occurs and sometimes one terminates a little sooner than the other. In lateral view the caeca show three large loops arching toward the dorsal surface. Between these, secondary loops appear. The three principal loops, while at first directed dorsally, ultimately curve with the body wall, and when viewed from above are seen to be turned also toward the median line. At these points the intestinal caeca approach each other very closely; in fact, the space between the most anteriorly located loops is very small. With this relation in mind it is evident that the digestive system is more extended than anticipated, and that each of the caeca if drawn out straight would reach nearly or fully twice the length of the entire body. There are also places where the intestine widens out considerably and shows distinct enlargements. In these places the diameter may become 0.68 mm., while towards the posterior extremity it is reduced to 0.225 mm.

We are not sure how far it is possible to compare our description of the alimentary system in *Paragonimus kellicotti* with that of the other forms given by previous writers. We are not unconscious that those descriptions are possibly less detailed rather than actually different. What is certainly significant in explaining some variations is that much in previous accounts is taken from a

study of specimens flattened under pressure. Such specimens must be badly distorted, since this worm has a thick fleshy body and is reduced by the pressure to a mere fraction of its normal thickness. Nevertheless we feel that a comparison is worth while, and yields some minor evidence that is significant and helpful in the solution of our problem. On the whole it may be said that the anatomical study of the alimentary canal in *Paragonimus westermanii* and *Paragonimus ringeri* reveals some small but characteristic differences from that of *Paragonimus kellicotti*. The pharynx is distinctly smaller than in the latter. In *Paragonimus westermanii* it averages 0.5 mm. long by 0.3 mm. broad, according to Kerbert; it is, then, not a spherical structure, as may be inferred both from these dimensions and from Kerbert's figure. The pharynx of *Paragonimus ringeri*, according to Leuckart, is spherical and 0.3 mm. in diameter, but Kubo gives it as elongate, measuring 0.4 mm. by 0.3 mm. The oesophagus is also shorter in both others than in *Paragonimus kellicotti*, being in *Paragonimus westermanii* 0.14 mm. long (Kerbert), and in *Paragonimus ringeri* 0.02 mm. long according to Leuckart.

Kubo was the first to see that the regions of the two branches immediately following the median oesophagus are identical histologically with that canal, and should be counted a part of it and not of the intestinal caeca which continue them but are so different in structure. He gives a length of 0.3 mm. for the undivided region and 0.2 mm. for the lateral branches. He comments on the sharp transition from the oesophageal to the intestinal region, and ascribes to the latter a diameter two to three times as great as we found in *Paragonimus kellicotti*. The peculiar condition shown in both these forms in that the intestinal caeca do not arise immediately from the end of the oesophagus, but somewhat further distad from the branches of the median oesophageal canal, is no doubt a generic character. It is well illustrated by the figure of this region which Kubo gives.

Some features of the intestinal caeca constitute possible differences. Kerbert says that in *Paragonimus westermanii* the intestinal caeca pass posteriad from the bifurcation of the oesophagus parallel with the surface of the body. They reach nearly to the posterior extremity of the body, and then end

blindly. He gives the length of the caeca at about 8 mm., or approximately the length of the whole body. This description is certainly at least incomplete, for the caeca in *Paragonimus westermanii* as he figures them do show some irregularities, and perhaps this condition varies with the age of the parasite in all species of this genus. But proceeding from the account of Kerbert, one may say confidently that such a simple relation does not exist in *Paragonimus kellicotti* or in *Paragonimus ringeri*. Leuckart shows, both in his description and by his sketches, that the intestinal caeca of *Paragonimus ringeri* are complicated by a number of loops and turns just as they are in *Paragonimus kellicotti*. Yet one point must be borne in mind, namely, that the latter parasite is the more attenuate. In it the intestinal caeca therefore do not lie very widely separated from each other, and where the major loops arch through the body towards the median dorsal line only a very small space intervenes.

One may ask if the formation of the loops is the same in both species. According to Kubo the course of the intestinal caeca in *Paragonimus ringeri* is entirely irregular and very varied. This condition is shown in his figures, and may be a real difference between that species and *Paragonimus kellicotti*. We hesitate to accept this interpretation, since his figures are drawn from much flattened preparations, and in such the general symmetry of the caeca which we have described is so much modified that it can rarely, if ever, be seen. It is possible that an examination of undistorted specimens of *Paragonimus ringeri* will show some regularity and balance in the course of the intestinal caeca. For the present one must accept the statement that in *Paragonimus ringeri* the course of the intestinal caeca is irregular and asymmetrical.

These relations between the species are expressed synoptically in the following table:

	<i>P. westermanii</i>	<i>P. ringeri</i>	<i>P. kellicotti</i>
Size of Pharynx ...	0.3 × 0.5 mm.	0.3 × 0.3 mm. or 0.4 mm.	0.44 × 0.44 mm.
Length of Oesophagus	0.14 mm.	0.2 or 0.3 mm.	0.21 — 0.25 mm.
Character of Intestinal Caeca	Relatively simple, little longer than body (?)	Looped irregularly (?) Twice length of body (?)	Looped symmetrically Twice length of body

### *The Excretory System*

The excretory system in *Paragonimus kellicotti* is relatively simple. The excretory pore is of such size (0.05 mm. in diameter) that under favourable conditions its position may be determined with the unaided eye. It lies on the dorsal surface 0.2 to 0.225 mm. from the posterior extremity, and opens into a small canal about 0.062 mm. in diameter and 0.225 mm. long. A cuticular layer lines the canal, surrounding which is a well-developed muscular layer. The canal passes directly forward, terminating abruptly approximately midway between the dorsal and ventral margins of the large excretory sinus which lies in the middle axis of the body. The greatest dimension of this sinus is in a dorso-ventral direction, in which also it approaches the body surface very closely, coming within 0.15 mm. dorsally and 0.315 mm. ventrally behind the acetabulum. It extends anteriorly to within 0.45 mm. of the branching of the oesophagus, tapering to its termination here gradually, and finally disappearing nearer the dorsal than the ventral surface. As already stated, the sinus lies in the median axis, but in the region of the uterus, which is located on the left side somewhat anterior to the middle of the body; it shows a distinct bending to the right. Its median position is again resumed beyond the uterus. This bending is undoubtedly conditioned by the increase in size of the uterus as it becomes filled with eggs.

The sinus has a distinct lining, as have also its main branches. These branches are very numerous, though along the sides of the sinus they are not conspicuous in the posterior region, but anteriorly in front of the acetabulum it is easy to see a number that are given off. These branches connect with the large stellate flame cells that are distributed throughout the body. We could not follow out the system to its ultimate details, but detected many flame cells in sections.

The excretory system of *Paragonimus westermanii* consists also of the prominent elongated central reservoir and numerous lateral branches; in general a similar condition exists in *Paragonimus ringeri* and, as we have shown, in *Paragonimus kellicotti*. Considered more closely, however, there are here also certain minor differences. According to Kerbert the large central reservoir in *Paragonimus westermanii* is located in the posterior part of the



body, and opens to the exterior through a circular opening at the posterior pole. Kerbert also does not mention the presence of a short duct between the sinus proper and the excretory pore. This may be a minor error in observation, or due to methods of technic. But the appearance of the reservoir in *Paragonimus westermanii* is in most cases, according to Kerbert, that of an elongate or pear-shaped tube, or it may also be a spherical bladder. The latter observation was made upon fresh material. The excretory bladder in *Paragonimus ringeri*, according to Leuckart, is a very much elongated narrow sinus with its greatest dimension in the dorso-ventral direction. A short canal leads from the posterior extremity of the sinus to the excretory pore. This opening has a diameter of 0.05 mm., and lies on the ventral surface. Our specimens of *Paragonimus ringeri* are slightly distorted, but seem to indicate this relation. In *Paragonimus kellicotti* the excretory pore appears on the dorsal surface in about the same relation with the posterior extremity.

Kubo locates the pore at the posterior end. He also describes the canal system as originating from only two large main canals which empty one on each side into the posterior portion of the reservoir. Other authors have seen many such lateral canals emptying into the central reservoir, and our findings in *Paragonimus kellicotti* agree with them.

The other relations are expressed in synoptic form as follows:

	<i>P. westermanii</i>	<i>P. ringeri</i>	<i>P. kellicotti</i>
Location of Excretory pore	Posterior pole	Ventral, near posterior extremity, or at posterior pole (?)	Dorsal, near posterior extremity

### *The Reproductive System*

With the exception of the vitellaria,\* the female reproductive organs of *Paragonimus kellicotti* extend only a little beyond the second quarter of the body. The genital pore lies about 0.078 to 0.104 mm. behind the acetabulum, usually a little to one side of the median line. This is the opening of the genital cloaca. To

\* In this discussion we have retained the classic names for the organs although, since the appearance of Goldschmidt's convincing demonstration, it can hardly be doubted that these designations are inappropriate and incorrect.

the right, and near the dorsal wall, is the ovary, while on the left, nearer the ventral surface, appears the highly coiled uterus. At about the level of the ovary, but in the median line, lies the shell gland, from which the proximal portion of the uterus emerges.

The genital cloaca is a short flask-shaped structure only 0.2 to 0.21 mm. long. It opens to the surface of the body through the genital pore, and receives the terminal ducts of both the male and the female reproductive systems. The wall of this structure consists of an inner homogeneous or granular layer, and an outer muscular wall which is composed essentially of circular fibres. The terminal portion of the vas deferens narrows to a small tube, the ductus ejaculatorius, which enters the genital cloaca at about the middle of the base. The metraterm, or terminal portion of the uterus, enters near the base, usually on the side opposite to the acetabulum. Cirrus and cirrus pouch are absent.

Stiles records the genital pore as 'median, right, or left, in specimens from hogs,' but in our specimens variance from the median location is slight, and where it is found, easily attributable to a slight distortion of the body surface. What Kubo calls the ductus communis genitalis evidently corresponds to what we have designated the genital cloaca. In our specimens it is certainly a distinct structure and not a common canal formed by the junction of the male and female ducts. It is very much smaller relatively than shown in his sketch, as is demonstrated by its length, which he gives as 0.4 mm. in *Paragonimus ringeri*.

The male reproductive system of *Paragonimus kellicotti* consists of two testes that occupy the third quarter of the body, two vasa efferentia leading from them, and the single terminal vas deferens. The vasa efferentia unite near the dorsal margin of the excretory sinus to form the vas deferens.

The testes lie one on each side of the body, and occupy nearly the entire space between the intestinal caeca and the excretory sinus in the posterior region of the body. The central portion of each testis is located approximately midway between the dorsal and ventral body surfaces. Their symmetrical arrangement is slightly disturbed, inasmuch as the right testis is usually a trifle posterior to the left testis. In this particular there is some variation, for in one specimen the right testis was found to lie anteriorly to the left.

This variation will be discussed later. The form of the organs is noteworthy (Pl. VII, fig. 4, also Pl. IX). Long slender lobes extend from the upper margin of the central mass; these are usually two in number. From the point of origin they arch upward and backward through the parenchymatous tissue. The terminal ends of the lobes are greatly enlarged, and frequently sub-divided into large rounded lobules. Other such lobes, three to four in number, extend from the ventral surface of the central mass. These connections are not as long as those given off from the dorsal margin, but their terminal ends are much more prominently enlarged, and almost always show two or more lobules heavier than those on the dorsal projections of the organ.

The vasa efferentia, which are two in number, corresponding one to each of the testes, have a diameter of 0.026 to 0.046 mm. at their point of origin. They spring from the middle portion of the testes at about the same dorso-ventral level, although the right one is a little longer than the left. Each after its origin ascends gradually, and at the upper margin of the excretory sinus they lie parallel to each other. At this point the right one crosses to the left side of the body, and after both descend somewhat, they unite to form the vas deferens slightly to the left of the sinus at a level just below the shell gland and vertically above the genital pore.

The vas deferens is at the start a relatively large duct, 0.062 to 0.1 mm. in diameter. It drops in general ventrad, arching first towards the anterior extremity. Keeping to the left side it approaches the acetabulum partly surrounded by the coils of the uterus. Then directing its course posteriad it circles close to the acetabulum towards the ventral surface, finally terminating in the genital cloaca. During its passage to the genital cloaca, the vas deferens shows a number of characteristic features. Just at the posterior margin of the acetabulum it suddenly narrows to a small tube, which becomes even smaller as the genital cloaca is approached. This portion of the vas deferens is heavily muscled, no doubt functioning as the ductus ejaculatorius. Surrounding the vas deferens in the region where it suddenly narrows is a mass of glandular cells (Pl. XI, fig. 21). These gradually disappear toward the genital cloaca and probably constitute the prostate gland. The inner cuticular lining of the

genital cloaca is continued into the ejaculatory duct, but farther on the nuclei of cells appear, although the lining retains its granular structure. A muscular layer consisting of circular fibres completes the wall of the vas deferens.

The testes of *Paragonimus westermanii* lie near the dorsal side of the body behind the transverse vitelline ducts (Kerbert). In structure they show five to six lobes. The right testis lies close behind the transverse vitelline ducts, while the left one is found nearer the posterior end of the body. For this reason it is possible to differentiate between an anterior right, and a posterior left testis which are distinct in Kerbert's figure. It must not be forgotten that this was drawn from a much flattened specimen. The position and relation of the testes is different in *Paragonimus ringeri* according to Leuckart. Here they lie, nearly symmetrically, well towards the posterior extremity of the body. They are not confined to the dorsal region, but occupy the greatest part of the space between the intestinal caeca and the excretory sinus. In the dorso-ventral dimension they have considerable extent. Comparing the figures given by Kerbert and by Leuckart, one sees a clear difference in form. The testes of *Paragonimus westermanii* are dense and the lobes more regular in form, while those of *Paragonimus ringeri* are diffuse and irregularly lobed.

Kubo compares the testes of *Paragonimus ringeri* to an outspread hand, and says they consist of four or five long lobes radiating from a common centre. Neither his description nor his figure will fit conditions in *Paragonimus kellicotti* as we have found them, but they agree more nearly with Kerbert's account of *Paragonimus westermanii*. Here again it is hard to say how much true conditions are modified by the distortion of flattened preparations, but the testes in *Paragonimus kellicotti* are very much larger and both the central mass and the more numerous lobes are larger and heavier than the same structures as figured in the other species.

Kerbert records that the vasa efferentia in *Paragonimus westermanii* pursue a dorsal course, arching over the transverse vitelline ducts; after several loops they approach the ventral surface and unite to form a common seminal vesicle which is continued into a short ductus ejaculatorius. The vasa efferentia in *Paragonimus ringeri*, according to Leuckart, pursue no such a

course, in fact neither one arches over the transverse vitelline ducts, while the left one drops gradually to the ventral surface without ascending dorsally. In addition, the vasa efferentia in *Paragonimus westermanii* are more slender, being 0.01 to 0.016 mm. in diameter (Kerbert), while in *Paragonimus ringeri* they are 0.045 to 0.1 mm. in diameter (Leuckart). We do not feel sure to what extent these supposed differences, which are in fact rather minute, depend upon trivial errors in observation or description or upon different conditions of contraction in the body, and how far they indicate real variations in structure between these closely-allied species.

As already stated, the vasa efferentia in *Paragonimus ringeri*, according to Leuckart, do not pursue a symmetrical course. It is important to examine this further. The right tube rises gradually toward the outer margin of the shell gland, crossing close under the transverse vitelline ducts, then drops almost perpendicularly, approaching at the same time the median line, and under the ventral margin of the shell gland unites with the vas efferens of the opposite side. This one pursues a much simpler course, for it does not approach the dorsal surface, but is directed downward toward the anterior extremity and median plane; it crosses the margin of the excretory sinus relatively far forward, and continuing its ventral and median direction is finally united with the other into the common duct.

This portion of Leuckart's description might be construed in either of two ways: (1) that the union of the vasa efferentia takes place ventrally to the excretory sinus, or (2) that it occurs dorsally to the sinus. The figure in the text illustrating this point shows the first relation, while the description might be understood as indicating either. Among the specimens of *Paragonimus ringeri* in the Ward collection there was one which had been broken just behind the genital pore. The anterior portion of this parasite was sectioned, and the relation of the vasa efferentia studied. In this specimen the ducts united dorsally to the excretory sinus. The vas deferens drops ventrally surrounded in part by the coils of the uterus. That there is opportunity for certain variation in the relation of the vasa efferentia, is readily understood from the fact that, with continued growth of the parasite, the uterus becomes

engorged with ova, finally pressing nearby structures out of their original relationships.

The vitellaria of *Paragonimus kellicotti* are very extensively developed. Not only do they cover the parasite laterally, but also extend over the dorsal surface of the body, meeting both anteriorly and posteriorly, and leaving but a very narrow space in the median line which becomes broader just in front of the transverse vitelline ducts, finally to disappear entirely toward each extremity, although around the oral sucker there is also a free space. The ventral surface presents a relation very similar to the dorsal, except that on this surface the vitellaria do not meet near the anterior sucker, and correspondingly do not approach the median line so closely. The product of the vitelline glands is gathered up by many small ducts, which gradually unite to form two main trunks on each side, one arising in the anterior region and the other in the posterior region. These converge toward a point a short distance in front of the middle of the body, and unite here to form the large dorsally located transverse vitelline ducts.

This distribution of the vitellaria stands in partial contrast with the condition in *Paragonimus westermanii* and *Paragonimus ringeri*. In these species, as described, a considerable space near the median dorsal and ventral line is not covered by vitellaria. In other words, the vitellaria on the dorsal surface of *Paragonimus kellicotti* approach more closely the median line than do those of *Paragonimus westermanii* and *Paragonimus ringeri*, and in other ways also appear to be more extensive in their development. The relation on the ventral surface is very similar. The vitellaria of *Paragonimus kellicotti* approach the median line more closely than those of *Paragonimus westermanii* and *Paragonimus ringeri*, but at the same time not so far as they do on the dorsal surface (Pl. VII, fig. 1).

The vitelline reservoir in *Paragonimus kellicotti* is a pear-shaped structure arising at the point of union of the transverse vitelline ducts. These ducts become considerably narrower just before terminating in the vitelline reservoir. At the point where they unite the reservoir has its greatest width; dropping ventrally for a short distance and at the same time becoming narrower, it directs its course anteriad. Having reached a plane just below the shell

gland, it changes its course, and proceeds slightly upward until about the level of the junction of Laurer's canal and the oviduct. Here it turns sharply to the right, and unites with the short canal formed by the union of these two ducts (Pl. X, fig. 20).

This condition closely typifies the relation in *Paragonimus westermanii*, but not that described by Leuckart and Kubo for *Paragonimus ringeri*. Here the two vitelline ducts unite to form a single canal, which drops ventrally a short distance, and then broadens out into a large flask-shaped reservoir 0.5 mm. long. From the median margin of the anterior extremity of this reservoir, and on the inner side of the unpaired canal, a small duct arises and passes dorsally to unite with the duct formed by the junction of the oviduct and Laurer's canal.

The ovary in *Paragonimus kellicotti* lies on the right side, close to the dorsal wall of the body. Only a small portion extends down far enough to lie alongside of the excretory sinus. The transverse vitelline ducts bound this organ posteriorly (Pl. IX, figs. 15, 16). In relative size the ovary is about as large as a testis, but not so diffuse. It presents a more compact form since, even though lobed, the lobes are heavy and do not extend so far from the main body of the organ as do those of the testes.

The oviduct is a short tube, at first relatively wide (0.36 mm.). It arises near the upper margin of the ovary, and from that portion which lies toward the median line. It soon narrows down to a very small tube, 0.018 mm. in diameter and about 0.13 mm. long. Just after its origin on the ovary, the wall of the oviduct becomes heavily muscled. This portion of the canal is the oöcapt. The oviduct rises slightly toward the dorsal surface, but drops again towards the plane at which it left the ovary. Here it unites with a small duct, which in fact is the so-called Laurer's canal. The wall of the oviduct consists of the cellular lining as is described for the male reproductive system, and the outer circular muscle layer (Pl. X, fig. 19).

Little can be said of these organs in a comparative way. The ovary lies in the same region of the body in all three species. We did not find any constant differences in its form or in the structure or relations of the oviduct in the different types.

Near the beginning of Laurer's canal is an expansion measuring

0.057 mm. in diameter, and from this pocket there extends outward to the right a blind pouch, or small seminal receptacle, about 0.195 mm. long and 0.052 mm. in diameter. Laurer's canal makes its way from this pocket in a sinuous course towards the dorsal surface. While at the expanded region the canal is relatively large, it soon narrows down to a very small duct. It proceeds in a large loop directed posteriad, followed by another small loop in the opposite direction to a short vertical stretch directly above the shell gland which terminates on the dorsal surface of the body in the region of the transverse vitelline ducts. The expansion and the seminal receptacle, as well as the entire lower portion of Laurer's canal, swarm with spermatozoa. The oviduct and Laurer's canal unite to form a tube about 0.031 mm. in diameter. This extends only 0.045 mm. before it receives the vitelline duct, and then widens to form the oötype. The oötype discharges into the proximal end of the uterus.

Stiles and Hassall (1900) have stated that a receptaculum seminis is lacking in the pig lung fluke. Their statement is not surprising in view of the real condition, which is shown at a glance in the figure representing the shell gland complex (Pl. X, fig. 20). It is not possible, so far as we can determine, to detect such a structure in total preparations, but sections through this region demonstrate beyond question the existence of a true receptaculum seminis in its normal location. It has the form of a blind pouch opening into Laurer's canal near the inner end of the latter organ. At this point the canal itself is much expanded, and the receptacle can hardly be said to possess a neck or duct. Consequently the cavity of the pouch is in constant and open communication with the lumen of the canal, and spermatozoa circulate freely in the common space. The receptacle is small, measuring at most 0.195 mm. in length by 0.052 mm. in maximum width, so that its extreme tip does not even reach the border of the shell gland.

The condition shown by the receptaculum seminis is of great interest from the standpoint of comparative anatomy. As is well known, there has been much discussion regarding the function and meaning of this organ. Most students regard it as a structure which is not of present functional value in any important way, at least among the trematodes, and certainly its variable character and



occasional complete absence are strong arguments in favour of such a view. In some cases Laurer's canal is reported to be lacking, and in others the receptaculum has been said to be wanting, as in the present instance. The actual presence of so insignificant a sac is only a theoretical correction of that statement. It may properly be designated a mere vestige of a structure about to disappear entirely. It is even possible, of course, that individual variation between different specimens is present to a sufficient extent to reduce it still further than the condition represented in the figure. So far we have found no evidence of its variation in size in *Paragonimus kellicotti*.

According to Kerbert, the lower portion of Laurer's canal in *Paragonimus westermanii* is provided with a seminal receptacle, which he figures somewhat larger than we find it in *Paragonimus kellicotti*. Leuckart, however, doubts the accuracy of this observation, since he did not find such a structure in *Paragonimus ringeri*. A seminal receptacle certainly is present in *Paragonimus kellicotti*, and Kerbert not only records its presence in *Paragonimus westermanii*, but also gives its measurements. If this structure is not present in *Paragonimus ringeri*, its absence may mark a characteristic difference in structure in the latter species. We are rather more inclined to believe that its presence can be demonstrated, and that if the three species differ at all in this respect, it will be found to be in the degree of development, or perhaps one should say, of reduction, which this organ manifests. Yet Kubo states that, despite zealous search, it was not possible for him to detect such a structure, and such a definite statement creates a strong presumption that the organ does not exist in *Paragonimus ringeri*.

The shell gland as reported for *Paragonimus ringeri* by Leuckart is a large organ, lying a little to the right of the median dorsal region of the body. It is 0.5 mm. thick and about 1 mm. long. This organ is also well developed in *Paragonimus kellicotti*. It lies close to the dorsal wall, and is more or less oval in shape, although somewhat irregular in outline, and measures 1 mm. long, 0.5 mm. thick, and 0.87 mm. broad. Kubo makes it slightly smaller, viz., 0.8 by 0.6 mm. Kerbert records approximately the same location in *Paragonimus westermanii*, although the shell gland

is distinctly smaller, being 0.2 to 0.3 mm. long by 0.12 to 0.14 mm. broad. The shell gland surrounds the proximal portion of the uterus, as well as the terminal portions of those ducts which go to form this part of the female reproductive system.

The uterus in a fully-matured parasite is a condensed, closely coiled tube. As the ova accumulate the uterus becomes widely distended, so that the coil occupies nearly the entire lateral portion of this region of the parasite. The walls of the uterus are made up of a relatively thin cellular layer, and a well-developed muscular coat consisting of circular fibres. Towards its terminal portion the uterus narrows down to form the metraterm. In this region the walls become heavily muscled (Pl. XI, fig. 22).

The relation of the uterine coils in *Paragonimus westermanii*, as indicated by Kerbert's sketch, is apparently simpler than is the condition in *Paragonimus ringeri* and *Paragonimus kellicotti*. In the former parasite the loops are open, and may be distinguished readily, but in the latter two forms they are close, and the entire organ presents the appearance of a solid mass. The age and stage of development must be a controlling factor in this condition, and very likely there is no constant difference here between the species.

It is well known that among the Trematoda one finds at times an exact reversal in the usual position of the organs, chiefly of the reproductive system, so that the specimen is a mirror image of the usual relation. Such a condition has been designated amphitopy, and occurs in some species so frequently that it is impossible to say which is the normal and which the reversed situs genitalium. In *Paragonimus* such a reversal has been observed by several investigators. Ward reported it for *Paragonimus kellicotti*, and we can confirm the record. In it the uterus lies on the left side, the right testis is anterior and slightly larger, the ovary is on the right side, and the bend in the excretory reservoir is to the right. This condition is not frequent in this species. Kubo found it in seven cases out of eighteen in *Paragonimus ringeri*. It is interesting to note that in the specimen figured by Looss (1914, p. 321) the organs are represented in what we regard as the reversed location, and not in the normal position. One figure published in Leuckart (1889) showed the same reversal. This has led to some confusion,

since the location of organs is stated by different writers in diametrically opposite terms.

As one of us (Ward, 1908, p. 178) has noted previously, the correct measurement of ova is not a simple matter. All sorts of direct errors in counting micrometer spaces and in computing actual values are not only possible but, in actual practice, frequent. They are not easy to test or detect. Against the danger of computing averages from a small number of specimens it is hardly necessary to warn the student, although this error has been committed by some experienced men. A more insidious error is caused by the unconscious selection of the larger and more conspicuous specimens; in this way an investigator may raise the true average considerably and reduce the range of size through the elimination of the smaller specimens. Such a tendency can be detected if the full series of measurements is given, but can only be inferred with some hazard if the extreme and average measurements are the only data printed.

The contrary difficulty, which is even more serious and more difficult to detect, will be introduced by that observer whose emphasis on the need of reporting every item he sees leads to his measuring and recording the size of absolutely every egg in a group. In any preparation one finds a considerable percentage of ova that are clearly abnormal. The shell is distorted by pressure or osmotic currents, which have made it over-large or over-wide; or in its process of manufacture by the parasite some interference with the normal course of events led to the moulding of an aberrant shell. The distorted form, or the atypical contents, demonstrate its unfitness for consideration in such measurements. To measure and record such an egg in the description of a species, unless the fact be given in connection with a note on the character of the individual egg, is usually to hamper, and certainly not to aid, the work of future students.

Here we may quote again on this matter the views of Looss expressed in an article in this journal (1907, p. 149) which I cited previously in my discussion of this topic. He says:—

‘There exist, of course, among the immense number of ova in an individual worm always some which are either larger or smaller than the rest, or even evidently misshapen. In my opinion, it is of no use to record carefully the measurements of these eggs also.

For the description and definition of a species it is much more important to select for measurement those ova which appear to be normal, and to present the size and shape typical for the species. It may be added in passing that young worms with few ova in their uteri usually do not afford normally-shaped and normally-sized ova.'

Finally it does make a difference what is the exact source of the eggs measured. Those which are taken from the body of the worm near the beginning of the uterus, i.e., which are just formed, do not agree in size and proportions with those at the end of the uterus ready to be laid, or those which are collected after deposition by the worm. I have noted on several occasions a tendency of the student to measure ova from the first coils of the uterus, because there they are less crowded, and hence more easily seen and measured. With the passage of the eggs through the uterus they increase in size, probably by imbibition of uterine fluid, and the increase seems to be most marked in case the ovum enters upon its development during the intra-uterine period, so that when deposited the egg shell contains a more or less advanced embryo.

Since one of us (Ward, 1908) has emphasized the significance of differences in the size of the eggs of *Paragonimus* as reported by various observers, especial attention was devoted to a study of these structures. An effort was made to get series of measurements under standard conditions, and to ascertain how far these varied from measurements previously given for eggs from similar sources. A start was made with *Paragonimus kellicotti*, and since it was evident at first thought that eggs taken from the body of the worm, or measured while still enclosed within it, might differ from those that had been laid in the normal manner, the first set of measurements was made from material that had been deposited naturally by the adult parasite.

The mucous exudate obtained from the bronchi and from the worm cysts in infected hog lungs was brought carefully into glycerine jelly and mounted within asphaltum rings. These preparations contained large numbers of ova, and being protected from any pressure by the cover-glass, the ova were in a natural, undistorted and undamaged condition.

Series of these eggs were measured, excluding only such

specimens as were distinctly aberrant in form, or bore evidence of having suffered some mechanical injury. The maximum length obtained was 0.0875 mm. and the minimum 0.0775 mm., with an average of 0.083 mm. The widest specimen measured 0.065 mm. and the narrowest 0.0525 mm., while the average width was 0.0559 mm.

The only other investigators who have measured these eggs under similar conditions are Stiles and Hassall, who state (1900, p. 603) that twenty-five eggs taken from cysts in the lungs of hogs varied in length from 0.096 mm. in maximum to 0.078 mm. in minimum, with an average of 0.0856 mm., and in width from 0.06 mm. in maximum to 0.048 mm. in minimum, averaging 0.0532 mm. It will be noticed that so far as the length is concerned the minimum measurement of Stiles and Hassall is practically identical with our minimum, but the maximum is nearly ten micra larger. We were unable to find any eggs of that length in material mounted so as to remain free from pressure, and it is clear that they recorded very few since their average size is only slightly larger than our record. The width given in their records does not vary greatly from that we record. The slight differences of from 3 to 5 per cent. in the averages of length and width can not be regarded as of serious significance. These figures represent, undoubtedly, the approximate dimensions of the egg of *Paragonimus kellicotti*.

In order to determine under similar conditions the size of the ova from the human lung fluke, slides were made in the same way, using the sputum obtained from an infected Korean. The material was not fresh as in the case of the hog parasite, but had been sent in formol from Chemulpo, Korea. A series of measurements from this material, after it had been treated exactly like the series of eggs from the sputum of the pig, gave the following values:—Length in maximum 0.097 mm., minimum 0.08 mm., with an average of 0.0872 mm.; breadth in maximum 0.055 mm.; minimum 0.046 mm., with an average of 0.0506 mm. Some months later we obtained through the kindness of Dr. M. M. Null, of Seattle, Washington, sputum from the patient, a Korean also, in that city who had been found to be infected with the lung fluke (see Null, M.M., 1910). Four series of eggs from this material, after treat-

ment in the same manner as before, were measured separately, with the following results:—

	Length in mm.			Width in mm.		
	Maximum	Minimum	Average	Maximum	Minimum	Average
Series (a) ... ..	0.0884	0.0754	0.0812	0.0546	0.0442	0.0493
Series (b) ... ..	0.0884	0.0780	0.0822	0.0520	0.0468	0.0499
Series (c) ... ..	0.0858	0.0780	0.0806	0.0546	0.0442	0.0496
Series (d) ... ..	0.0858	0.0780	0.0813	0.0520	0.0468	0.0483
General Average ...			0.0812			0.0492

These series of eggs of the human lung fluke from different localities do not agree perfectly in measurements. This is especially noticeable in the length, which in the material from Korea had a much higher maximum, and consequently an average about 7 % greater. The width is almost identical, as the range agrees perfectly, and the average of the one differs only 2 % from that of the other. Furthermore, it will be noted from the measurements cited that the range of variation in width is slight, being much less than the range of variation in length. It should be noted, as explained later, that the form of the egg is identical in the two cases. By reason of the extreme care used in obtaining these measurements, we believe they represent closely the true dimensions of the egg of this species and the probable range of size in normal undistorted eggs.

There are many other observations on the eggs of the human lung fluke. In 1880 Baelz reported that the bodies he found in sputum measured 0.13 by 0.07 mm. This record may be rejected as unquestionably erroneous since it does not agree with any other report, and more especially since it was not cited three years later by the same author, who then gives the size of the ova as 0.08 to 0.1 by 0.05 mm. He gives no average size, but this range is close to that we obtained (0.08 to 0.097 by 0.05 mm.) for the Korean material. One other record is given for eggs taken fresh from

human sputum. Manson (1882) records the average size of these ova as 0.085 by 0.051 mm.

Other records concern measurements made from preserved material or that of which the condition and method of handling is not stated. The most aberrant of these comes from Yamagiwa (1890), who measured in sections of the human brain and lungs ova which he attributed to the lung fluke, and found them in one case from 0.04 to 0.064 mm. by 0.024 to 0.04 mm., and in the other from 0.049 to 0.06 mm. by 0.029 to 0.036 mm. These measurements are so extreme that they can not be accepted as belonging to the same species as that from which the other measurements were taken. On the other hand, they agree more closely with the eggs of *Schistosoma japonicum*, although smaller even for that species than the size given by others. Now *Schistosoma japonicum* has been discovered since Yamagiwa's work was published, and was not taken into account in his discussion. Without entering here upon any discussion of the other species, one may say confidently there is every reason for eliminating his record from the category of cases assignable to *Paragonimus*.

Several other observations which undoubtedly concern the lung fluke remain for consideration. The record of Leuckart (1889) may have been made on material from the tiger or on some from the human host. He gives the length as varying from 0.08 to 0.1 mm., which agrees almost exactly with our figures, and states the width at 0.056 mm. which is barely larger than our recorded maximum width, 0.055 mm. These are distinctly larger than the figures given for ova of the tiger lung fluke, and hence probably were taken from specimens of the human lung parasite. Katsurada (1900) found the ova of the human lung fluke to vary from 0.0875 to 0.1025 mm. in length with an average of 0.0935 mm., and from 0.0525 to 0.0663 mm. in breadth with an average of 0.057 mm. Mackenzie (1904) records the length as 0.0855 to 0.0997 mm. with an average of 0.0913 mm., and the width from 0.048 to 0.069 mm., averaging 0.0552 mm. Both the extremes and the averages of these two records depart rather widely from our observations, and we are unable at this time to suggest any explanation for the discrepancy.

Kubo did not devote particular attention to the egg, as he

measured only ten specimens and gave but a very brief description. According to his records, the average length is 0.07 mm. and the average breadth 0.06 mm. These figures do not agree even approximately with those of any other investigator, and show a shell relatively much shorter and broader than anyone else has found. In fact, this average length is less than the minimum figures given in any previous paper, while at the same time the average breadth is greater than any other average, and equal to the maximum for this dimension given previously.

These various results obtained from the measurements of eggs demonstrate wide discrepancies in technic or some inaccuracy in methods. That the eggs of *Paragonimus* really vary as much as these figures seem to indicate is hardly a tenable hypothesis. Looss, whose accuracy is unquestioned, says of these different records that he has personally found, in undistorted worms, eggs only from 0.077 to 0.081 mm. long and from 0.046 to 0.05 mm. broad, and accordingly the variant records undoubtedly belong to another species or to much distorted eggs in which the cover is easily forced off by pressure. We also have been unable to find any such variation in normal eggs as these figures given by different writers seem to indicate, even when the eggs are taken from adults in different hosts or from widely separated regions. Hence we are forced to assume the introduction of errors of some sort.

In general it appears from these data that the eggs of *Paragonimus kellicotti* are broader and shorter than those of *Paragonimus ringeri*. These differences are not pronounced, and are certainly not sufficiently clear to allow of their use in the differentiation of species, as in extreme cases the sizes overlap. There is, however, a characteristic difference in form which may be seen on comparison of the non-operculated ends of the shell. In *Paragonimus kellicotti* that end tapers off rather sharply so as to produce the effect of a pointed extremity. In *Paragonimus ringeri* the corresponding end of the shell has a wider curve, due to a greater flare in the sides, which gives the egg a more pronounced elliptical outline. While this difference is trifling it is so distinct that once learned the student can readily pick out the species on comparison of the eggs. It was noticeable, for instance, that the two sets of ova from the human lung, discussed on pp. 141-2,



differed somewhat in length from each other, but agreed perfectly in the outline of the non-operculate end of the shell, so that one of us measuring them at once recorded this correspondence in his notes before the data had been compared with other records or any attempt made to interpret them.

Finally, it is noteworthy that the various records of size indicate much greater differences in length than in width, and there is a possibility that the eggs do vary widely in length while retaining a more uniform standard width. One could readily frame a hypothesis of egg formation which would agree with the known structure of the organs and the possible uniformity in width together with variation in length; but at present these supposed facts are too uncertain to be used in such a manner.

The eggs of the Philippine forms were very carefully measured by Musgrave (1907, p. 35), using a Zeiss photomicrographic apparatus. In length they vary from 0.062 to 0.098 mm., with an average of 0.074 mm.; and in breadth from 0.047 to 0.063 mm. with an average of 0.057 mm. Compared with our own measurements it will be noted that the maximum length is in exact agreement, but the minimum and the average length are far too small. It may be, as Looss has suggested, that Musgrave has found and measured some eggs of *Schistosoma* due to an intercurrent infection. The elimination of these lower values will bring the minimum and the average into agreement with our figures. But the width of the eggs as reported by Musgrave is open to the contrary objection. The minimum value agrees, but the maximum is far beyond what we found. This, of course, brings the average up above our record. It is only just to call attention to the fact that the figures of Musgrave for the width of the eggs agree fairly with those of the same dimension given by Katsurada and Mackenzie, although his values for the length of the eggs are very far below theirs. The evidence is inadequate for a final decision regarding the Philippine form.

The eggs of the form found in the tiger measure 0.08 by 0.045 mm., according to Kerbert; and Looss, who apparently re-measured the eggs of the same specimens sent to Leuckart, gives the dimensions as 0.077 to 0.081 mm. long by 0.043 to 0.05 mm. broad. - These figures are practically identical, and as far as they

go indicate a slightly smaller egg than is found in the other two species. We did not have material for a re-examination and comparative study of these structures. The difference indicated, even if fully corroborated by a re-study of the material, is too slight to serve as a diagnostic basis for the species, since as in the case of the other species maximum values for *Paragonimus westermanii* exceed minimum values for *Paragonimus ringeri*. The same is true so far as length is concerned for *Paragonimus kellicotti*, but the egg of the latter species is always distinctly wider than the maximum value obtained for *Paragonimus westermanii*. The comment of Kerbert, not shown in his figures, that the eggs of *Paragonimus westermanii* are slightly flattened at the [both ?] poles, seems to furnish a slight difference in the form of the egg between this and the other species.

#### GENERAL DISCUSSION

##### *The Genus Paragonimus*

Looss was the first to give an extended description of the genus, and his diagnosis slightly modified is reproduced by Stiles and Hassall (1900, p. 563). The description was so successfully written that even an extensive study of these three species has disclosed only minor corrections. Despite their distinctly insignificant character it seems worth while to call attention to these characters. The pharynx, while well developed, manifests a tendency for a spherical form rather than an elongate, and even in the extreme case does not depart much from the spherical. In our opinion the oesophagus should not be designated as *very* short. It always appears in total preparations to be shorter than it actually is, since it rises obliquely towards the dorsal surface and thus is foreshortened in any longitudinal aspect, while in many specimens this inclination is so emphasized by contraction of the body that the branching of the caeca rests directly upon the pharynx and thus appears to originate from the latter without the intervention of any median unpaired region which is the oesophagus. The failure to see the oesophagus in total preparations is often responsible for the belief that such a region is non-existent when in fact it is well developed. In such cases it shows up

conspicuously when the living worm stretches the anterior end, and becomes apparently obliterated when the worm assumes an average position or is contracted even moderately. In these instances the true form and relations of the oesophagus may be made out in lateral view, which one rarely gets of a trematode either naturally or in preparations, and may also be determined from a study of serial sections. From these it appears that the oesophagus suggests often the form of a letter S placed vertically, since it starts posteriad from a ventrally located pharynx, curves well to the front, and then turns again towards the posterior end to join the caeca. The beginning of these branches lies longitudinally so close to the pharynx that one does not see the vertical separation and the contracted or twisted oesophagus that joins them.

Looss states that one testis lies obliquely behind the other, and this is hardly correct in any sense. As we have shown, the central mass of the one organ is located directly opposite that of the other, and the only difference is found in the lesser development of the anterior lobes in one, usually that on the right side. This is undoubtedly due to the fact that the total mass of the uterus which lies just anterior to that testis is greater than that of the ovary which is anterior to the other. When ovary and uterus are reversed, as has been reported to occur in exceptional cases, then the testes show the reverse of the usual development in that the anterior lobes of the left testis are less prominent than those of the right testis, and the organ appears to be located slightly posterior to that on the other side. The testes are in fact opposite each other and one appears slightly anterior to the other only because of the relative development of the anterior lobes.

Both Looss, and Stiles and Hassall after him, emphasize the absence of a receptaculum seminis. As has been already shown in the discussion, this organ is present even though it be only poorly developed, and the proper form of statement would emphasize this fact.

As noted above, all of these points are distinctly secondary, and the genus description remains substantially as outlined by Looss. On the other hand, one can hardly agree with him in regarding the genus as nearly related to the *Fasciolinae*, especially to *Fasciolopsis*, as he maintains it to be. Since we have just

learned through personal correspondence that Professor Odhner has in print a discussion of this matter, it seems best to omit here any elaboration of this topic.

Stiles and Hassall (1900, p. 604) include in the genus two other species as follows: (A) *Paragonimus rudis* (Diesing, 1850) from the lung of a Brazilian otter; (B) *Paragonimus compactus* (Cobbold, 1859) from the lung of the Indian ichneumon. Neither of these has been reported since it was originally found, and of neither have we been able to secure material for comparison.

### *Cuticular Spines*

It is important to point out more precisely the significance of our discovery concerning the cuticular spines. They constitute in our opinion a most convenient and accurate criterion for the distinction of species, and one which may well be applied to other genera among Trematoda to the advantage of the taxonomist on the one hand, and of the practitioner and pathologist on the other. To the latter, desirous of making a rapid and accurate diagnosis of a form which falls into his hands, such a definite feature will be of marked value. This is especially true since no complicated and time-consuming technic and no array of apparatus are demanded for the determination. It is sometimes possible to tear off with fine forceps a piece of the outer skin with some of the spines *in situ*; it is always possible to slice off freehand with a razor a thin layer of surface tissue containing them. Such a fragment, mounted roughly, gives a good view of the spines, and thus affords means for diagnosing the species once that the precise character of the spines has been determined for that species. This is pre-eminently a method for diagnosis from fresh material, since, as is well known, in many cases the spines are caducous in life and are easily lost also if the specimen lies some time in a preserving fluid. In fact, spines disappear or are overlooked so easily that many investigators have paid little attention to the statements regarding their presence or absence given by previous writers, knowing that the previously described specimens or their own might easily have suffered the loss of these structures and yet be in good condition for the determination of other structural features.

We should not neglect to say that in our experience these characters cannot be determined in specimens that have been reduced to series of sections. Several such series of *Paragonimus* have been loaned us by colleagues for the purpose of testing the character of these structures, and in no such case have we been able to determine anything concerning the precise form or the arrangement of the spines, even after long and painstaking effort. Undoubtedly such a series might contain a bit of cuticula, removed tangentially from a region where the spines were preserved well, that would give the desired evidence, but after our experience we are inclined to regard such an occurrence as exceptional, and to consider serial sections as unfitted to furnish the data desired regarding the cuticular spines.

We have not been able to find record of a single species in which the precise form and distribution of these cuticular spines have been worked out. Yet they evidently possess advantages afforded by few structural features among the trematodes for the precise comparison of closely related forms. The great difficulty in comparing flukes results from the soft and variable form of most organs and the absence of hard parts. Descriptions are couched in terms that vary from individual to individual, and measurements in a given species range beyond the extremes of others, both among those truly similar and related and also among those that actually are more distant. The size of the suckers, which has been selected as one of the usual measurements in specific descriptions, varies considerably with the contraction of the specimen, and is moreover not always easy to determine, while it undoubtedly increases with the age of the specimen, although to what degree has not been fixed. Similar difficulties in the case of other organs contribute to make the description of the average trematode a recital of general, generic and even family characters rather than of specific features.

Various investigators have sought to utilise the ova as characters for specific differentiation. The difficulties involved in placing dependence upon this factor are well exemplified by the discussion of the lung flukes in the preceding pages. Some important deductions have been made correctly on this basis, but the procedure is dangerous, as is any such argument based on a single factor. Furthermore, Looss has shown clearly that there are two species

of *Clonorchis* in man, and yet the eggs are hardly distinguishable in size or form.

The demonstration of another anatomical feature composed of material that cannot be altered by pressure, contraction, or other mechanical influence, and relatively constant in form among different individuals, furnishes a diagnostic element of distinct importance. When our attention was first drawn to the cuticular spines, and they seemed to furnish such an element, we felt the matter deserved the most careful study before any announcement was made. By the work reported in this paper one may fairly claim that a demonstration has been given for the essential similarity, even though not for the absolute identity, of these spines in different regions of the body of a single worm, and also on different individuals in the same host and in different hosts. We have been able to recognize a graduation in size and frequency characteristic of different parts of the body of the worm, and also a variation in size in different worms which may be due to the difference in host that harboured the specimens studied, but is more likely attributable to differences in age and growth of these structures in different individuals. In spite of the extreme differences in size and frequency, there is no approachment between the species, but rather added emphasis upon their real distinctness.

In our opinion, these spines will furnish convenient and precise specific distinctions between still other species of Trematodes, and further studies to test this view are now in progress. It may be pointed out that this would be a most natural condition. Cuticular outgrowths, such as hairs, spines, scales, and other processes of varied form, have long been utilized by systematists in other groups as a means of identifying species, and have proved to be convenient and accurate characters for the description and differentiation of species. In animal groups which have been the object of long and intensive study, and in which taxonomy may justly be said to be more securely established than in groups of more recent study and hence less perfectly known, the use of such characters as specific criteria is generally approved. Even more than that, it may be said the species are distinguished on the basis of such differences when otherwise the structure, so far as worked out, is only known to be identical. Under these circumstances, we do not think we are

venturing on dangerous or untried ground in maintaining the specific integrity of these three different forms of lung fluke, when the contention is supported by differences as clear and unmistakable as those we have demonstrated between the spines. The larger, more striking morphological differences, which some years back were regarded as specific in value, are now generally accepted as of generic or family rank. The proper evaluation of those items which are confessedly insignificant will yield a firm basis for the proper conception of species among the lower forms. Nowhere is there more need of such careful analysis of minor features than among parasitic worms, where great confusion reigns by virtue of the rapid and inexact treatment that has been accorded these forms in the past. Many investigators of the present day have broken away from that unfortunate tendency, and are analyzing the structure of such forms with great care. We hope that the factor to which we have called attention so prominently in this paper may prove to be of wider usefulness than in this case merely. Even should that not prove to be true, we are still confident that species of *Paragonimus* can be readily and accurately determined by means of the cuticular spines. Such a precise determination is of evident general interest, since only by it can be determined the range of territory infected by a definite form, the number of different species that threaten man in a given place, and ultimately the measures that must be taken to eliminate these parasites from the list of the enemies of man.

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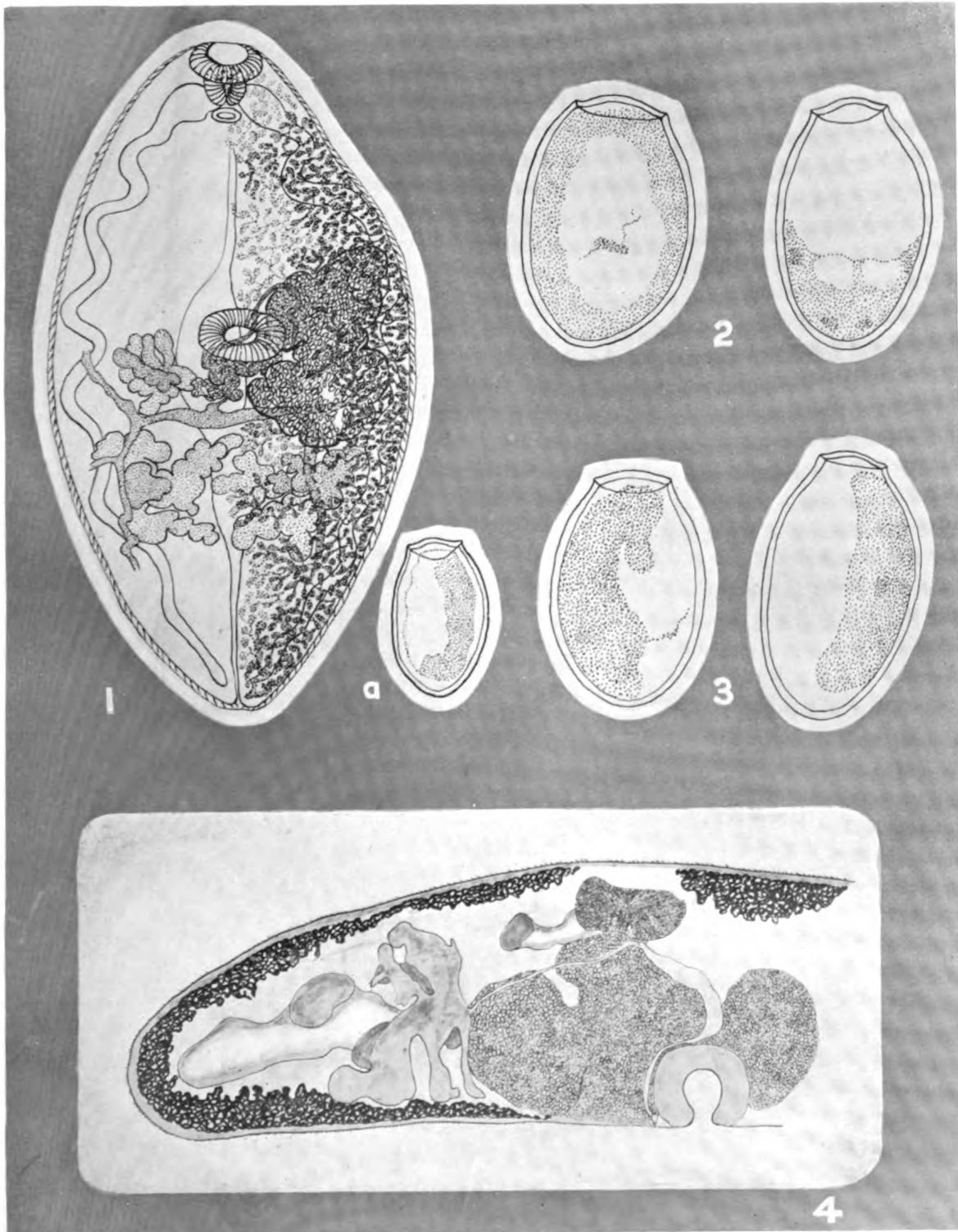


## EXPLANATION OF PLATES VII-XI

All figures are made from camera drawings of microscopical preparations, unless otherwise stated.

## PLATE VII

- Fig. 1. *Paragonimus kellicotti*; total preparation seen from the ventral surface. The vitellaria are represented on the left side of the worm, and omitted from the other side in order to show ovary, testis, vitelline ducts, and intestine normally obscured by them. The specimen had been stained, flattened under pressure, and mounted.  $\times 7.5$  diameters. a Egg from same specimen.  $\times 300$  diameters.
- Fig. 2. Eggs of *Paragonimus kellicotti* showing ordinary variations in form. These specimens were taken from mucus of lung.  $\times 475$ .
- Fig. 3. Eggs of *Paragonimus kellicotti* taken from uterus of parasite.  $\times 475$  diameters.
- Fig. 4. Reconstruction of a series of sagittal sections of *Paragonimus kellicotti* showing the left half of the body in the posterior region as seen from the median sagittal plane. One can distinguish readily the marginal vitellaria, the dorsal shell gland with a bit of the main yolk duct or yolk reservoir, the uterus massed around the acetabulum, the irregularly-lobed testis with its vas efferens joining the vas deferens, and finally the extreme posterior tip of the intestinal caecum of that side.







## PLATE VIII

Figs. 5-11. Spines of *Paragonimus kellicotti* to demonstrate the substantial identity of these structures from different regions of the same specimen and on parasites of the same species obtained from different hosts.

Fig. 5. From dorsal surface behind oral sucker.

Fig. 6. From centre of dorsum.

Fig. 7. From dorsal surface near posterior extremity.

Fig. 8. From ventral surface between oral and ventral suckers.

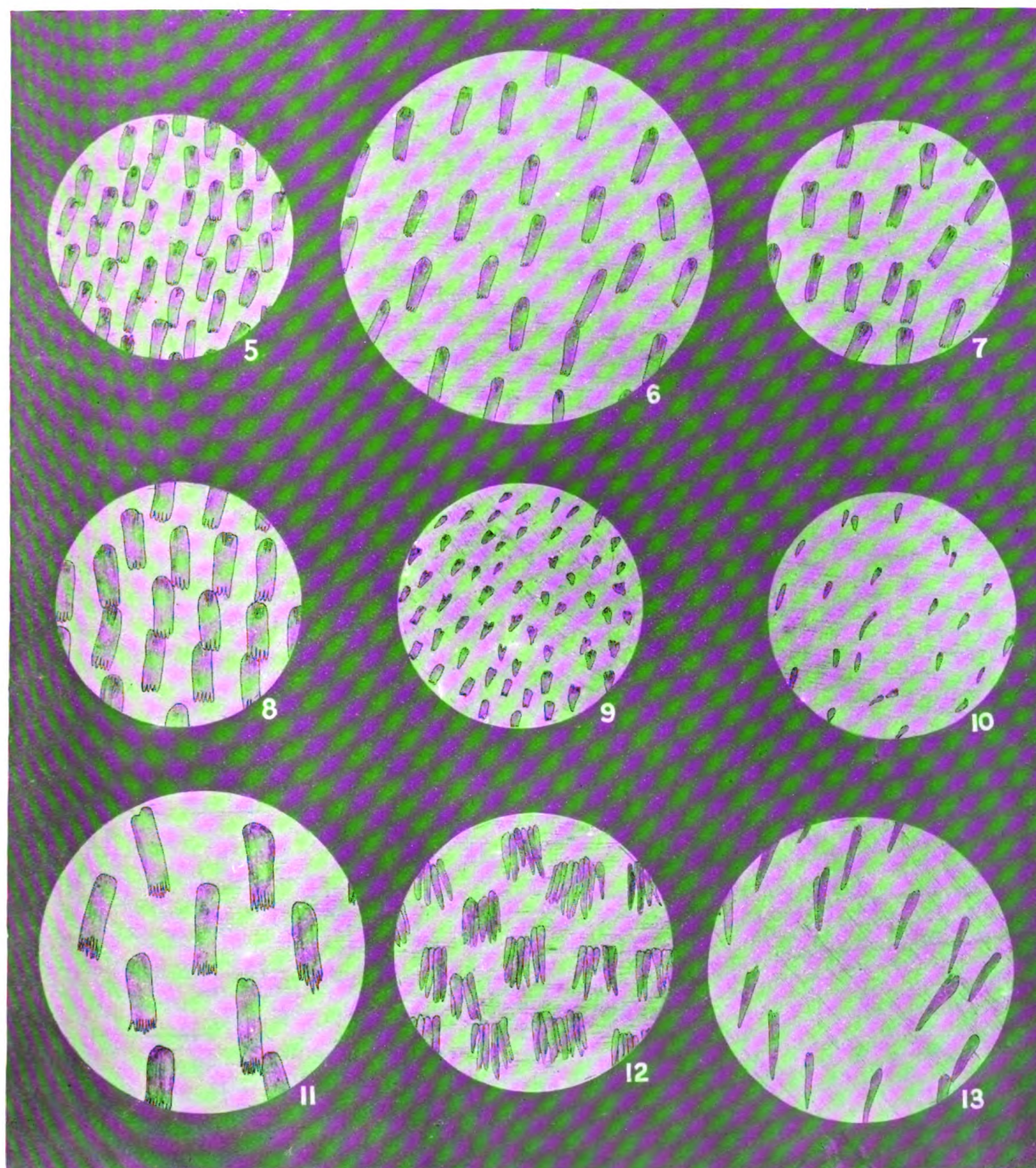
Fig. 9. From region near oral sucker.

Fig. 10. From region close to ventral sucker.

Fig. 11. From specimen taken from lung of cat.

Fig. 12. Spines from cuticula of *Paragonimus ringeri* from lung of man in Japan.

Fig. 13. Spines from cuticula of type specimen of *Paragonimus westermanii*.









## PLATE IX

Reconstruction of a specimen of *Paragonimus kellicotti* from the pig. The alimentary and reproductive systems alone are represented. The vitellaria are omitted and also the uterus, except in fig. 15, where it is outlined in part. The specimen was carefully preserved, and showed no evidence of distortion. It had not been flattened under pressure, as have most specimens drawn for illustrations in the texts. Consequently it shows more accurately the true form and relations of organs.

Fig. 14. In lateral aspect, showing the right side of the body only.

Fig. 15. In lateral aspect, showing the left side of the body only.

Fig. 16. In dorsal aspect, showing both sides of the body.

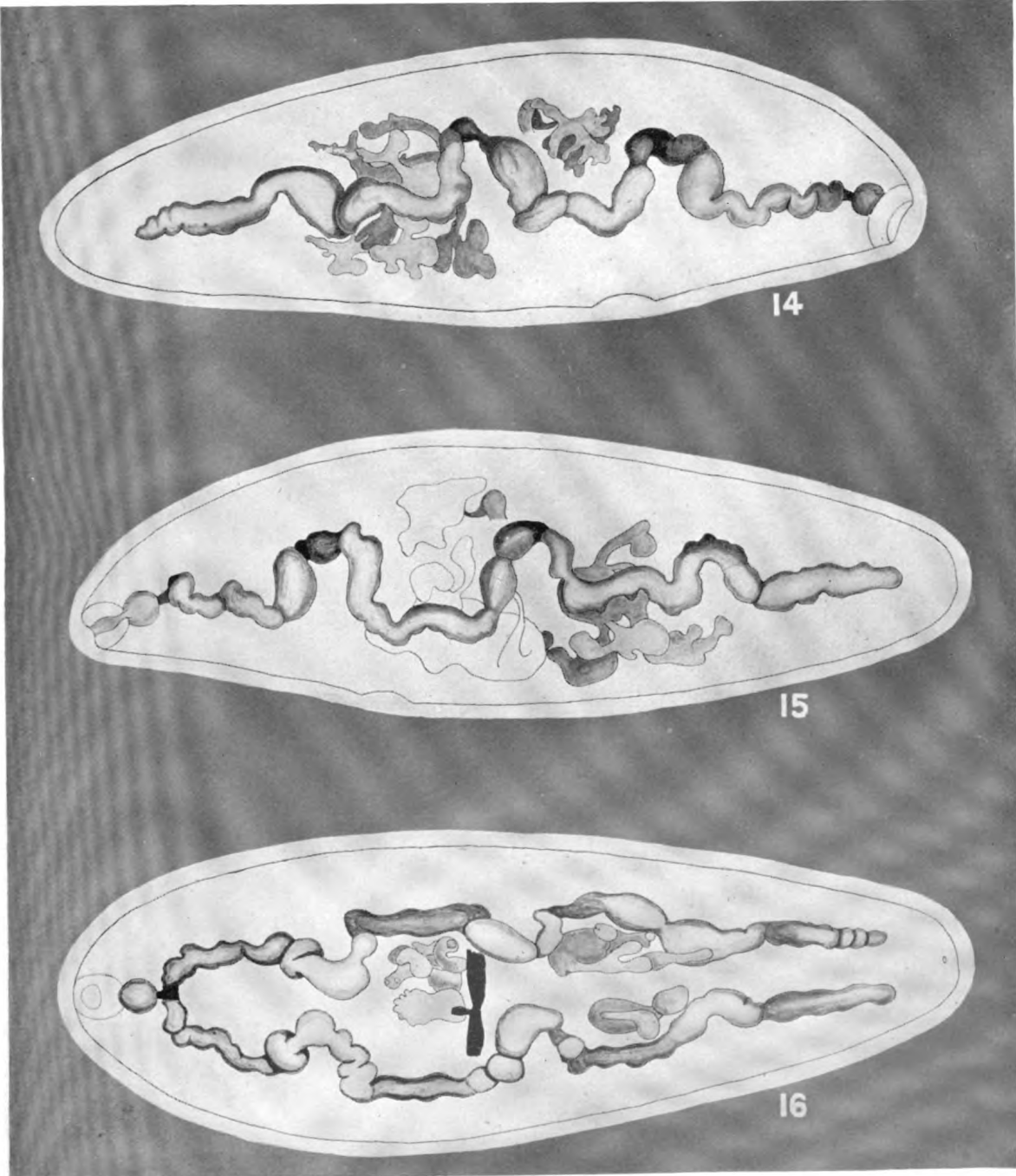




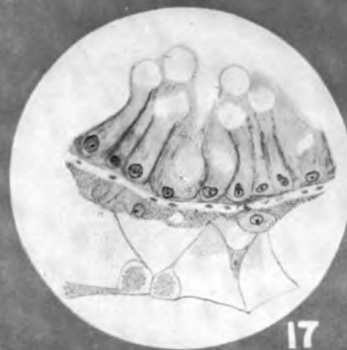


PLATE X

- Fig. 17. Cross section of intestinal wall, showing character of epithelial cells and subjacent tissue.
- Fig. 18. Cross section of body wall, showing cuticular spines *in situ*. The anterior end of the body lies to the right in the figure.
- Fig. 19. Section through ovary and oviduct, including also some cells of shell gland and the wall of the seminal vesicle.
- Fig. 20. Sagittal section supplemented from adjacent sections to show shell gland complex, including vitelline reservoir and common yolk duct, oviduct, seminal vesicle, Laurer's canal, oötype, shell gland, and beginning of uterus.



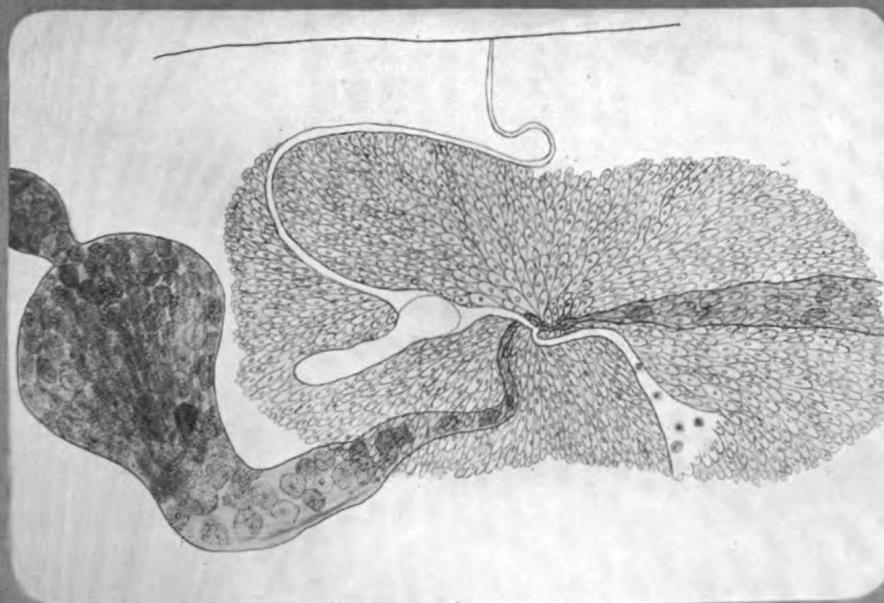
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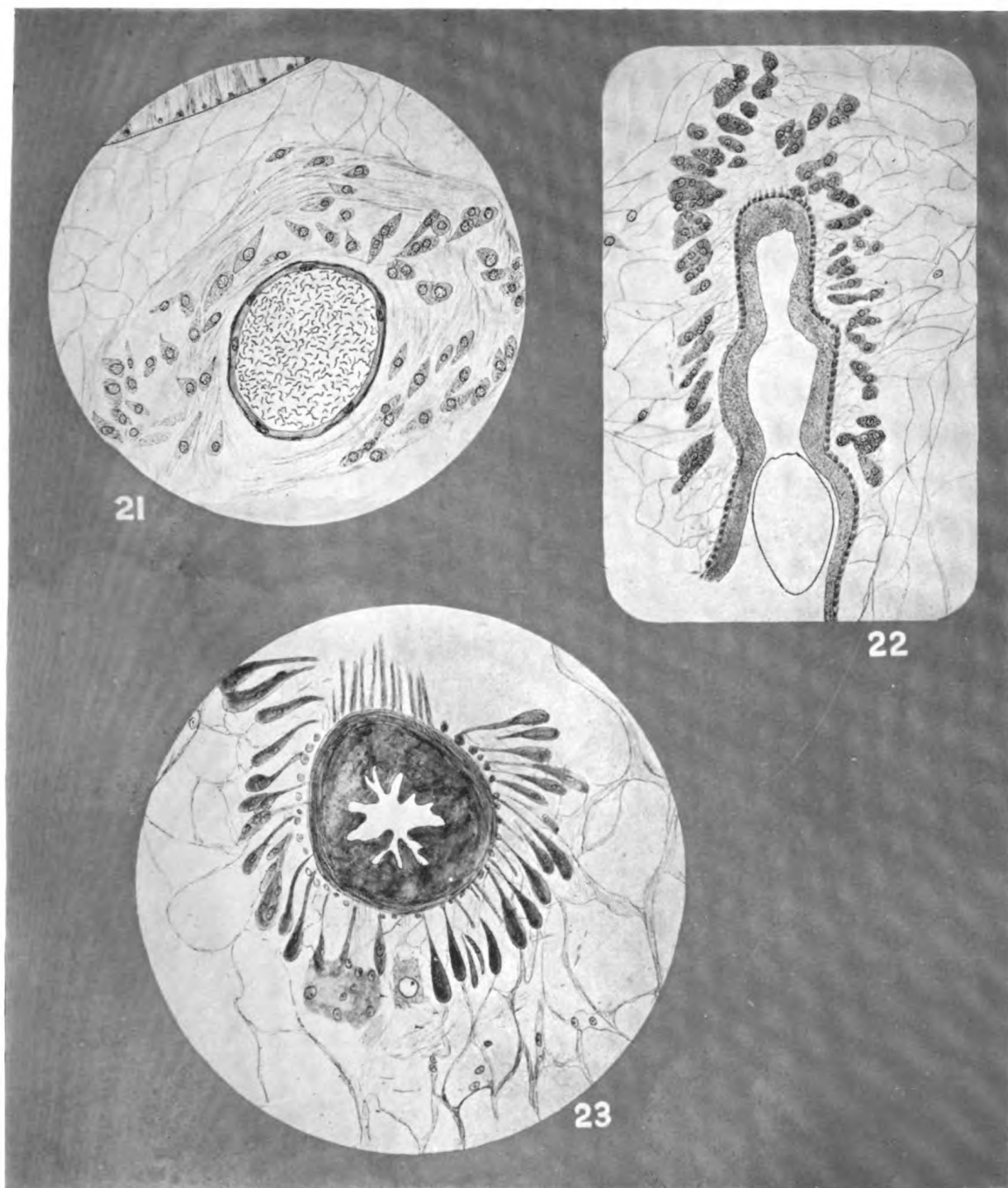






PLATE XI

- Fig. 21. Cross section of vas deferens near acetabulum. In this region the tube is filled with spermatozoa and surrounded by a loose mass of prostate gland cells.
- Fig. 22. Frontal section passing obliquely nearly through the length of the metraterm, which here contains a single egg. The layers of the wall and adjoining gland cells are clearly shown.
- Fig. 23. Cross section of oesophagus, showing layers in wall and associated salivary (?) gland cells.





## PRELIMINARY NOTES ON THE MOSQUITOS OF KABINDA (LOMAMI), BELGIAN CONGO

BY

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Kabinda is a healthy station situated at an altitude of about 2,800 feet. Malaria is known to exist, but is somewhat rare and of little importance. The habitations of the whites are grouped on the summits of several hills which are separated by ravines of varying depths. There are no swamps in the immediate neighbourhood of Kabinda, and mosquitos, in general, are relatively rare. They are, however, very sensitive to seasonal variations, and are more numerous at the beginning and end of the rainy season, when the rains are not excessive.

At the time of my first visit to Kabinda I caught on myself, in the doctor's quarters, a specimen of *Stegomyia fasciata*, the presence of which species had already attracted my attention. Afterwards, in addition to several 'ordinary' Culicine mosquitos, I captured a yellowish species (*Taeniorhynchus*) and an Anopheline (*Myzomyia*). Thus from the beginning I observed that, in spite of the relative rarity of these flies at Kabinda, representatives of different groups were present, and that, therefore, an investigation would probably be of value.

Returning later in order to pass some months at Kabinda, I settled down to the task of breeding these insects. There was no difficulty in obtaining the larvae, since it was only necessary for me, or a trained helper, to make a tour of the European or certain native compounds. Here would always be found the 'reservoirs containing stagnant water,' so strictly proscribed by malarial prophylaxy, namely, all kinds of receptacles containing rainwater or water intended for pigeons, poultry, ducks, etc. These receptacles, which are scarcely ever emptied, sometimes swarm with mosquito and Chironomid larvae.

Within a few months I bred a great number of mosquitos, several thousands, but before enumerating the tribes and important genera obtained (I am not sufficiently competent to determine with certainty the 'smaller' genera) I wish to make the following statements:—

(1) I have not been able to breed certain species which, however, occur in the houses of the station. On the other hand, I have obtained in this way a mosquito which I have never seen in, or even near, the houses. Thus, the species of *Taeniorhynchus* are quite common in the houses, and I have seen them every evening in my own; nevertheless, I have not bred a single specimen. The same may be said for the members of the genus *Mansonioides*, which, though very rare, are also to be met with in the houses of Kabinda, and yet have never been bred. This probably means that the larvae of *Taeniorhynchus* and of *Mansonioides* require a special type of stagnant water, probably swamp water, and are not able to live in the more or less filthy water of the receptacles occurring near human habitations.\* A few analogous facts which I have observed in other localities tend to strengthen this hypothesis. In the proximity of the station Mutombo-Mukulu there are numerous marshes, and mosquitos are very prevalent.

During my first visit to this station, the mosquitos captured in my temporary habitation belonged almost exclusively to the genus *Mansonioides*. At the time of my second visit (the station had in the meantime been removed to a plateau a short distance further from the marsh), the mosquitos taken in the house were nearly all the yellowish *Taeniorhynchus*. During this second sojourn at Mutombo-Mukulu, however, I bred a certain number of mosquitos, and yet did not obtain a single specimen of *Mansonioides* or *Taeniorhynchus*. The same facts were observed at Samba (Kasonga Niembo). On the other hand, I have succeeded in

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\* The immature stages of *Mansonioides* have been found by Dr. Ingram, in the Gold Coast, in borrow pits overgrown with a water plant (*Pistia stratiotes*). The larvae remain constantly below, attached, by means of their peculiarly modified siphon tubes, to the roots of the plant; the pupae, although not remaining continuously below, also attach themselves to the plant, by grasping the roots with their extremely elongate air-trumpets, and apparently pass considerable periods in this manner. The larvae of no African species of *Taeniorhynchus* have yet been discovered, but the life-histories of three or four American species have been worked out and are very similar to those of *Mansonioides*.

raising a few examples of *Megarhinus*,\* having found a few larvae in a receptacle, although these mosquitos are not seen in the proximity of habitations. This enormous mosquito, with the bent proboscis, does not occur in the houses, because it does not bite. But the fact that I have found larvae (four) of *Megarhinus*\* in a small receptacle containing dirty water and that these larvae produced adults, shows that this mosquito has no need of special water or of any particular water-plant.

(2) Generally speaking, the larvae of different species are not found in the same receptacle in any great variety. Those of a genus, or even of a species, not only predominate, but often are the only ones present at a given moment, and therefore the contents of a receptacle provide, as a rule, only a single species at a time. The same receptacle has furnished on one day specimens almost entirely of *Culex pipiens*,† and eight days afterwards almost exclusively of *Stegomyia fasciata*. This is probably due merely to the chance of oviposition.

(3) The Anophelines are relatively rare at Kabinda, both in the adult stage in and near houses, and in the larval stage, in artificial receptacles containing stagnant water. Among the thousands of mosquitos which have been bred, only a few dozen were Anophelines; these were obtained sometimes as rare examples among Culicines, and on one occasion as a pure 'culture' (associated Chironomid larvae having been removed).

Among the Culicines, two important genera, *Culex* and *Stegomyia*, constituted 90 per cent. of those found.

#### DETERMINATION AND CLASSIFICATION OF MOSQUITOS CAPTURED AND BRED AT KABINDA

Not being a specialist, I have had and still have great difficulty in determining the species bred, all the more so since I have been unable to consult the special works on the subject. The exact identification is rendered still more difficult owing to the recent changes, wrought by Mr. Edwards, in the classification and terminology proposed by Prof. Theobald. As I wished above all to make this work of value, I resolved to avoid doubtful examples,

\* The tribe or sub-family *Megarbinini* is intended, not the genus *Megarbinus*. H. F. C.

† Probably *C. duttoni*. Theob. H. F. C.

and have, therefore, confined my determination to specimens which I recognised. I have sent the others for identification to Mr. F. W. Edwards (British Museum) and to Prof. R. Newstead and Mr. H. F. Carter (Liverpool School of Tropical Medicine), and have asked them at the same time to revise my determinations. I shall, therefore, be able at a further date to complete and correct this small preliminary study.

The *Culicinae* (true mosquitos) are divided into four tribes—*Megarhinini*, *Sabethini*, *Culicini*, and *Anophelini*—and representatives of each have been found at Kabinda.

(1) *Megarhinini*. In a receptacle containing water for the use of pigeons, I discovered, on one occasion, among some Culicine larvae, four enormous larvae which, from a distance, resembled small fish. Although I had never seen any so large, I immediately surmised, from their size, that they were probably the larvae of a Megarhine. Such an opportunity being so rare, I decided to preserve a larva and pupa; on the appearance of one of the latter a larva was killed, and when a second pupa developed one of these was also killed. The two remaining larvae hatched into two superb Megharines, a male and a female, of the same species, viz.: *Toxorhynchites brevipalpis*, Theob. The pupal stages of these two mosquitos were of considerable duration, and occupied five days in the case of the female, eight days in the male, whereas this period in other mosquitos is usually only one or two days.

(2) *Sabethini*. These mosquitos were found several times in very small numbers among Culicines; twenty in all have been bred out, and two specimens were taken in the doctor's house. The characteristics of the larvae were not observed; all belonged to the same species—*Eretmapodites chrysogaster*, Graham.

(3) *Anophelini*. As previously mentioned, the Anophelines are uncommon in this district; the few dozen examples raised from larvae, and the few rare specimens captured, belonged to the genus *Myzomyia*, Blanch. The species obtained at Kabinda and at all the other localities examined in this region—Mutombo-Mukulu, Samba, Labefu, Pania, etc.—proved to be *M. costalis*, Loew.

(4) *Culicini*. This is the commonest tribe of mosquitos but the richest in genera and species, containing species of very variable size and colour. In consequence, it is the most difficult group to



study, and the most intricate as regards classification. I have so far observed representatives of six different genera of the tribe, but have only been able to determine four with certainty.

(a) Genus *Culex*, Linn.; *C. tigripes*, Grand.\* Five examples of this species have been bred, the large larvae being associated with those of other species of *Culex*. *C. pipiens* group†—at least 50 per cent. of the mosquitos which I bred belonged to this group.

(b) Genus *Mansonioides*, Theob. As previously mentioned, the larvae of these mosquitos have not been found, but I have captured a few adults of *M. uniformis*,‡ Theob., in the houses.

(c) Genus *Taeniorhynchus*, Arr. A score of adults were taken in my house, although none were bred. All the examples are of the same species, but show slight variations—*T. aurites*, Theob.§

(d) Genus *Stegomyia*, Theob. After *Culex*, my breeding experiments have furnished me chiefly with members of this genus; and almost all my specimens have been found, on very careful examination, to belong to the famous species, *S. fasciata*,¶ Fab. *S. fasciata* is very common here, and occurs both in and around the houses, chiefly towards evening, at which time, after sunset, they seem to be very active. The following curious fact may be suitably mentioned here. The mosquitos found in my house belonged, almost without exception, to the two genera *Taeniorhynchus* and *Stegomyia*. Now while the members of the genus *Stegomyia* became active and commenced to bite about sunset, it was almost exclusively those of *Taeniorhynchus* which annoyed me later, between seven and eight p.m.; before this they scarcely ever appeared.

Besides the four more important genera dealt with above (and which are of interest in tropical medicine) I have bred numerous specimens of two other genera of Culicines. Since I am in doubt

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\* Var. *fusca*, Theob. H. F. C.

† Judging from the species present in the collection forwarded by Dr. Schwetz, it would seem that this term refers to *C. duttoni*, Theob., *C. univittatus*, Theob., and *C. invidiosus*, Theob.; the true *C. pipiens*, Linn., was not represented. H. F. C.

‡ Only a small number of specimens referable to this genus has been received from Dr. Schwetz, some of which may be *M. africanus*, Theob. Owing to their poor condition, however, it is impossible to identify them with certainty. H. F. C.

§ The examples forwarded proved to be *T. cristatus*, Theob., not *T. aurites*, Theob. H. F. C.

¶ Other species of *Stegomyia* present in Dr. Schwetz's collection were *S. africana*, Theob.; *S. apicoargentea*, Theob.; *S. simpsoni*, Theob.; *S. poweri*, Theob. H. F. C.

regarding these, I have sent the specimens for determination to persons more acquainted with the subject,\* and shall refer to them in my next paper. A certain number of *Chironomidae* (other than *Ceratopogon*) and a few *Psychodidae* (other than *Phlebotomus*) have also been bred, the larvae of these flies being found with those of the mosquitos. I shall mention these insects again later (as well as their larvae and pupae) when the specimens have been determined.

My collection of mosquitos has been divided into four parts and sent to the School of Tropical Medicine, Brussels, the Museum of Tervueren, the British Museum and the Liverpool School of Tropical Medicine.

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\* These specimens are referable to the species *Culicomyia nebulosa*, Theob., and *Ochlerotatus (Protomacleaya) alboventralis*, Theob. H. F. C.

# ON THE PECULIAR MORPHOLOGICAL APPEARANCES OF A MALARIA PARASITE

BY

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## PLATE XII

In a paper entitled 'A new Malaria parasite of man' (1914), I described under the name *Plasmodium tenue* what I took to be a new species of malaria parasite. The peculiar morphological characters described were new to me, and I could find no illustrations or description of similar appearances.

Since writing the above-mentioned paper I have received through the kindness of Dr. Le Fanu, W.A.M.S., from the Gold Coast, a blood slide in which he thought he had recognised the forms I had described as *P. tenue*.

Dr Le Fanu had put this slide aside for further study, as he had noted before my description of *P. tenue* that there was something peculiar about the morphology of the parasites. The film was made from the blood of a native child that came for treatment. The infection is not a severe one: it is easy to find fields without any parasites. The forms in Dr. Le Fanu's slide are, however, in some respects even more peculiar than the *P. tenue* forms.

Firstly, I would point out that the blood cells present no evidence whatsoever of stretching or distortion, and the same peculiar forms of parasites occur in all parts of the film, thick and thin.

Secondly, there are present large forms of quartan parasites which appear to me to be *quite normal*. In the slide it is possible to trace a transition from normal ring forms to those in which *chromatin particles or strands without any protoplasm* occur in the red cells. A better idea of these will be got from the accompanying plate than from any description however detailed.

Three views seem to me possible as to the nature of these forms.

(1) That they are a new species of parasite.

(2) That they are degenerative, i.e., formed in the body under unknown conditions, and so perhaps analogous to the so-called quinine forms of parasites.

(3) That they are artificial, i.e., formed outside the body under unknown conditions.

The fact that among the parasites figured, forms are found in which chromatin alone without any protoplasm occurs in the red cell, is I think in favour of, but not decisive for, one of the latter two views rather than the first. The fact that normal quartan parasites are present is against these two latter views. It should be noted also that forms consisting of protoplasm alone, without chromatin, were not seen. Nor were pigment grains apart from parasites seen on the red cell.

While this paper was passing through the press, Balfour and Wenyon (1914) published a paper entitled 'The so-called *Plasmodium tenue* (Stephens).' In it they express the opinion that I had 'not produced any evidence to prove that he [I] was not dealing with an amoeboid sub-tertian parasite.' The fact that no observer (with perhaps one exception unknown to me when I wrote) since Laveran's discovery of the malaria parasite in 1880, i.e., during a period of thirty-five years, had described such amoeboid forms of the malignant tertian parasite, appeared to me to be very strong evidence against this view.

It is common knowledge that the malignant tertian parasite is to a certain extent amoeboid, but this is a very different condition from that I described in *P. tenue*. The authors quote for instance Ziemann, but a reference to his coloured plate will make it evident that he was not describing *P. tenue* forms. With regard to the possible exception I mentioned, I much regret that I had quite overlooked the existence of Dr. Balfour's plate published in 1908 and reproduced in the paper cited above. To Dr. Balfour certainly belongs the credit of the first description of markedly amoeboid forms of malaria parasites (other than the simple tertian), but a careful comparison of my plate illustrating *P. tenue* with that of Dr. Balfour will show that although there is a resemblance, it is not a close one. Now Ziemann's plate was published in 1906, and if extraordinary amoeboid activity of the malignant tertian parasite was already recognised it seems hardly necessary for Dr. Balfour to have published his plate of 'curious amoeboid forms' in 1908.

But to avoid controversial matter, let us now consider the substance of Balfour and Wenyon's paper. They figure two parasites, the first of which (in a single film from West Africa) is almost certainly identical with *P. tenue*, and possibly, too, this is true of the second parasite (from Bagdad). In this latter case, several films were taken just before death and an hour previously; all contained the same forms (Dr. Wenyon in a private letter). As to the nature of these parasites the authors appear to be in doubt, for in one part of their paper they state that 'it may be possible to find for these variations some mechanical explanation,' and in another that they are 'young parasites' (of *P. falciparum*) 'which are particularly amoeboid for some reason not clearly understood.'

Craig (1914), on the contrary, states that he 'is satisfied that *Plasmodium tenue* is an atypical form of *Plasmodium vivax*.'

It is perhaps not out of place to mention here that Emin (1914) has described a new malaria parasite under the name *Plasmodium vivax*, var. *minuta*.

When we have evidence as to what the nature of the forms I have described in this paper may be, and particularly whether they do or do not belong to the large quartan forms, we shall then probably be in a better position to estimate whether my view that *P. tenue* is a new species of malaria parasite is right or wrong.

It is of interest to record the result of an examination of further films from the case of *P. tenue*. Two films (March, 1914) showed quartan, and in one of the films a single pigmented (presumably simple tertian) parasite was found with the pigment in the form of rods, in an enlarged cell showing Schüffner's dots. Three films (June) showed quartan, and one (July), quartan. Young ring forms were not found in any of the films.

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EXPLANATION OF PLATE XII

The figures were drawn with an Abbé camera lucida at a magnification of 2300 (approximately).







# ON SOME PREVIOUSLY UNDESCRIBED TABANIDAE FROM AFRICA

BY

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*(Received for publication 1 February, 1915)*

## PLATE XIII

The flies considered in this paper belong to the genera *Tabanus* and *Haematopota* of the sub-family *Tabaninae*; with one exception they were obtained either from the Transvaal or from the West Coast of Africa. Those from the Transvaal were all taken in the vicinity of Onderstepoort, near Pretoria, and formed part of a collection which also included the following species: *Tabanus sericeiventris*, Loew, *T. insignis*, Loew, *T. taeniola*, P. de B., *T. ditæniatus*, Macq., *T. atrimanus*, Loew, *Haematopota scutellaris*, Loew (?), *Chrysops stigmatalis*, Loew, and *Diatomineura aethiopica*, Thun.

The types and co-types of the various species and of the variety herein described are in the collection of the Liverpool School of Tropical Medicine.

Genus TABANUS, Linn.

*Tabanus triquetroratus*, n.sp.

Medium-sized species with the dorsal surface of the thorax and abdomen chocolate-brown in colour; anterior half and posterior border of thorax, including whole of scutellum, pale greyish; hind margins of abdominal tergites with narrow pale borders expanding in the central region of the second, third, fourth and fifth visible segments into conspicuous greyish triangles and laterally into somewhat irregular pale areas. Eyes bare. Legs dark, the tibiae almost entirely white or creamy white. Wings faintly and uniformly tinged with brown.

*Head* (fig. 1): *Face*, jowls and posterior region steel-grey, clothed with rather long white hairs; sub-callus (denuded) shining dark-brown. *Front* very narrow, from eight to nine times as long as the width at the base; yellowish-brown in colour, somewhat darker at the vertex, and clothed with short yellow and black hairs. *Frontal callus* shining, dark-brown, rectangular, reaching from eye to eye, and with a linear prolongation in the central line extending slightly further than the middle of the front.

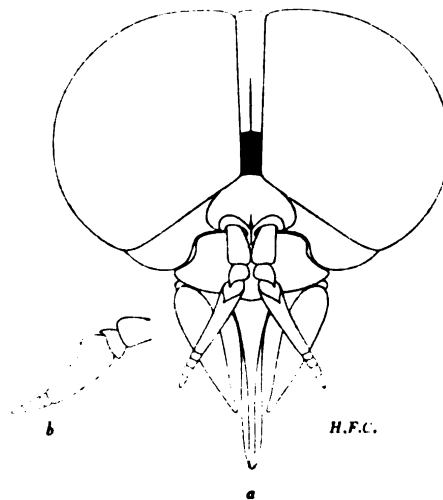


FIG. 1. (a) *Tabanus triquetrorhatus*, n.sp., front view of head, female. ( $\times 13$ , about).  
(b) Antenna of *Tabanus triquetrorhatus*, n.sp., female. ( $\times 13$ , about).

*Antennae* (fig. 1b) brownish-drab, darker, almost black, at the tips; first joint clothed with long white and short black hairs; second joint with a few white hairs ventrally and numerous minute black ones fringing the apical margins; third joint somewhat elongate with the dorsal process moderately pronounced and bearing a few short black hairs. *Palpi* creamy white, clothed with white hairs which, on the outer side of the terminal segment towards the apex, are intermingled with short black ones.

*Thorax*: Anterior portion of dorsum, as far as the transverse suture, ashy-grey, clothed with yellow and black hairs, the former apparently\* more numerous laterally; remaining portion of dorsum

\* The dorsal surface of the thorax is partially denuded in this specimen.

(except area immediately in front of scutellum, basal half of post-alar calli and *scutellum*, all of which are yellowish-grey) of a chocolate-brown colour; humeral and post-alar calli bearing long white hairs. *Pleurae* steel-grey, slightly darker anteriorly with whitish hairs.

*Abdomen*: Dorsal surface chocolate-brown, clothed with short black hairs; distal margins of segments, triangular areas and lateral expansions light grey, with yellowish hairs; pale triangles on second, third and fourth segments large, their apices reaching almost to the basal margins of the segments on which they are situated, that on the fifth segment considerably smaller and only half the height of the segment. *Venter*, two basal segments entirely grey, clothed with white hairs; remaining segments chocolate-brown with pale grey hind margins expanding somewhat laterally, clothed with white hairs interspersed on the darker areas, with black ones which become longer and more numerous on the last two segments.

*Wings*: Faintly brownish; veins and *stigma* pale reddish-brown. *Squamae* chocolate-brown. *Halteres*, stems and knobs brown.

*Legs*: Coxae grey, clothed with white hairs. Femora black, more or less pruinose ventrally and laterally, especially those of the hind legs; clothed with black hairs which are intermingled with, or replaced by, white hairs on the pruinose areas. Tibiae, except extreme apices, white or yellowish-white clothed with white hairs. Apices of tibiae and whole of tarsi black, with short black hairs.

*Length*, 12 mm.; length of wing 10.25 mm., width of head 4 mm.; width of front at vertex 0.5 mm.

*Habitat*: Calabar, Southern Nigeria; Dr. T. B. Adam, 1913; one female.

This species is evidently related to *Tabanus argenteus*, Surcouf. It is easily distinguished, however, by its general paler coloration, paler wings, and by its abdominal ornamentation.

*Tabanus fuscipes*, Ric., var. *oculipilus* n. var.

Closely resembling the typical form, but with the eyes more thickly covered with longer hairs, the femora distinctly paler and the basal portion of the third joint of the antenna somewhat shorter and broader. The femora are yellowish, only the basal third of

the front pair and the extreme bases of the middle and hind pairs being dark. The recurrent veinlet from the upper branch of the cubital fork is usually considerably longer and more conspicuous than in the typical form.

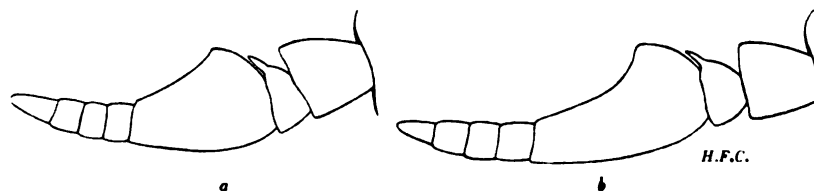


FIG. 2. (a) Antenna of *Tabanus fuscipes* var. *oculipilus*, n.var., female. ( $\times 35$ , about).  
(b) Antenna of *Tabanus fuscipes*, Ric., female. ( $\times 35$ , about).

*Length*, 11.25 to 13.25 mm.; length of wing, 8.75 to 10.25 mm.

*Habitat*: Onderstepoort, Transvaal; type female, 2.2.1913, Mr. G. A. H. Bedford; thirteen females, presented by Mr. F. V. Theobald, taken in the same district during January, February and March, 1911.

Both *Tabanus fuscipes*, Ric., and its near relation *T. ditæniatus*, Macq., belong to the sub-genus *Atylotus*, Osten-Sacken, all the members of which possess pubescent eyes. In these two species, however, the hairs are almost microscopical, whereas in the new variety the pubescence of the eyes is very marked and clearly visible under a pocket lens ( $\times 15$ ). Taking into consideration also the other differences mentioned it would seem that the form in question is worthy of varietal rank. *T. taeniztus*, Macq., which also occurs in South Africa and possesses hairy eyes, might perhaps be confused with this insect but may be distinguished, *inter alia*, by the presence of two sharply defined whitish, admedian, stripes on the thorax, by the abdominal stripes being white instead of yellowish, and by the legs and antennae being darker.

#### Genus HAEMATOPOTA, Meig.

##### *Haematopota transvaalensis*, n. sp.

(Plate XIII, fig. 3)

Medium-sized blackish species with conspicuous, pale, more or less circular spots on each abdominal tergite, grey, clearly-marked wings and dark legs—the front tibiae strongly incrassate.

*Head* (fig. 3): *Face*, jowls and posterior region whitish, clothed with long white hairs; upper part of face with a row of four velvety-black spots extending horizontally just below the bases of the antennae; each of the inner spots is rounded and is situated immediately below one of the antennae, while each outer spot is more or less triangular with its base touching the eye; face above the line of spots yellowish-brown with numerous, irregularly distributed, small black spots. *Front* rather dark mouse-grey, except the extreme lateral margins and round the lateral frontal spots, where it is much paler and almost white; vertex yellowish-brown, but with a short, narrow, greyish stripe in the middle line

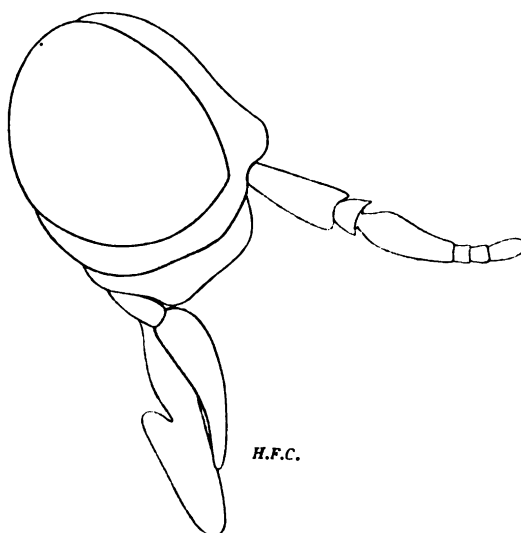


FIG. 3. *Haematopota transvaalensis*, n.sp., side view of head, female. ( $\times 18$ , about).

extending to the median frontal spot; front clothed with black and white hairs, the latter present on the paler areas. Lateral frontal spots large, rounded, touching the eyes; median spot small and inconspicuous. *Frontal callus* shining, olive-brown, of moderate depth and extending from eye to eye; spot below callus black, or nearly so, and conspicuous. *Antennae* (fig. 3) comparatively long and slender; first joint cylindrical, dark greyish-brown, clothed with short black hairs; second joint and extreme base of third, yellowish-brown, the former clothed with minute black hairs; third joint, except extreme base, very dark brown, terminal annuli almost

black, the last annulus about the same length as the two preceding together. *Palpi* grey; terminal segment clothed with short black hairs on outer side, replaced towards the base by a few rather long whitish hairs.

*Thorax*: Dorsal surface dark brown with three longitudinal light grey or whitish stripes; median stripe narrow, more clearly defined anteriorly, extending from front margin to scutellum (excepting a short distance in the centre where it is obliterated) and dilating somewhat posteriorly; lateral, admedian, stripes broader, terminating just above the inner extremities of the transverse suture, below each of which is a conspicuous, more or less triangular, white spot; side of dorsum and humeral calli darker grey, crescentic marks on hind margin sharply defined, whitish, each with the base of an ill-defined, moderately large, smoke-grey triangular area immediately above its outer extremity. *Scutellum* dark brown; the front margin yellowish-brown in the centre merging into light grey laterally, sides and broad median stripe, which extends to hind margin, grey. Dorsum sparsely clothed with glistening white hairs, humeral calli with long white hairs, post-alar calli with white and black hairs intermingled, and scutellum with hairs similar to those of the main portion of dorsum. *Pleurae* light grey, clothed chiefly with white hairs.

*Abdomen*: Dorsum dark brown, the lateral borders of the segments grey and the hind margins narrowly creamy-white; each tergite with a pair of comparatively large, yellowish to grey, admedian spots, and the third to sixth tergites each with a narrow grey median line; yellowish-grey hind margin of second tergite produced into a forwardly directed yellowish triangle. Dorsum clothed with black or brown hairs on the dark areas and with glistening white hairs on the remaining portions. *Venter* steel grey, with hind margins of segments paler, clothed with glistening white hairs; last segment darker with some erect black hairs.

*Wings*: Grey with rather fine pale markings as shown in Plate XIII, fig. 3; rosettes well-defined, apical sinuous mark single, extending from costa in first submarginal cell (where it is expanded) to wing margin at a point just below the lower branch of the third longitudinal vein, distal angles of third and fifth posterior cells, each with a large pale blotch. *Stigma* conspicuous,

dark brown with the proximal third paler; veins brown. *Squamae* pale. *Halteres*, stalks cream-coloured, knobs dark brown.

*Legs*: Coxae dark grey (upper half of front pair paler), clothed with whitish hairs. Femora mostly pruinose, clothed with white and black hairs, the former preponderating except on the front femora where the black hairs are more numerous (especially at the apices) and rather longer. Front tibiae black, distinctly swollen, with a conspicuous creamy-white ring at the base, middle and hind tibiae blackish-brown, each with two cream-coloured rings, hind pair slightly dilated; clothed with black hairs except on the pale bands where white or creamy-white hairs are present—on the hind tibiae the pale hairs extend, for some distance, on to the dark space between the rings. Tarsi black or dark brown, the first tarsal segments of the middle and hind pairs of legs mostly cream-coloured.

*Length*, 10 mm.; length of wing, 9 mm.; width of head, 3.5 mm.; width of front at vertex, 1.1 mm.

*Habitat*: Onderstepoort, Transvaal; one female presented by Mr. F. V. Theobald, 24.3.1911, and one other female by Mr. G. A. H. Bedford, 9.12.1912.

*H. transvaalensis* does not seem to resemble closely any of the species described by Loew in his work on South African Diptera, but is apparently related to *Haematopota masculosifacies*, Aust. From this, however, it may be distinguished by its larger size, paler scutellum, differences in wing markings (*inter alia*, the apical sinuous mark), and in its abdominal ornamentation.

*Haematopota theobaldi*, n. sp.

(Plate XIII, fig. 4)

Medium-sized chocolate-brown coloured species with reddish-brown wings; dorsum of thorax paler with three longitudinal smoke-grey stripes, dorsum of abdomen darker with a conspicuous ashy-grey median stripe and traces of spots on, at least, the apical segments. First antennal segment much swollen and shining black. Legs dark, the front tibiae incrassate with one pale ring, mid and hind tibiae with two pale rings.

*Head* (fig. 4): *Face*, jowls, and basioccipital region light grey, clothed with whitish hairs; upper part of face with a transverse row

of black spots, as in the preceding species, but less distinct, the inner ones, below the antennae, reduced to mere dots, the outer ones narrowly separated from the eyes. *Front*, clothed with glistening cream-coloured hairs, dark mouse-grey in the centre, light grey at the extreme lateral margins and round the lateral frontal spots;

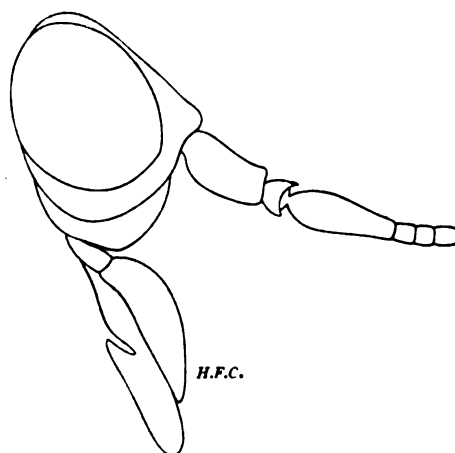


FIG. 4. *Haematopota tibobaldi*, n.sp., side view of head, female. ( $\times 18$ , about).

vertex with a large dark brown blotch, which becomes somewhat yellowish-brown at the margins, divided in the central line by a narrow grey stripe reaching to the median frontal spot. Lateral frontal spots, more or less circular, not in contact with the eyes; median frontal spot much reduced and inconspicuous. *Frontal callus* shining black, of moderate depth and extending from eye to eye; upper margin slightly produced in the centre, lower margin concave for some distance on each side of the middle line; spot below callus velvety black. *Antennae* (fig. 4) dark, moderately long; first joint strongly incrassated and shining black (greyish pollinose at base and on inner surfaces), clothed with sparse black hairs; second joint dull reddish-brown, upper angle somewhat produced, clothed with black hairs; third joint dark brown, slightly paler at extreme base, with the terminal annuli black—last annulus not quite so long as the two preceding taken together. *Palpi* dark grey; first segment clothed with white hairs, second segment clothed on the outer side with short black hairs interspersed with rather longer white ones towards the base.



*Thorax*: Dorsum chocolate-brown with three longitudinal, rather dark smoke-grey lines; median stripe narrow, paler at extreme anterior end, and extending to the scutellum; admedian stripes shorter, widening and becoming contiguous with median stripe anteriorly, terminating immediately behind the transverse suture as a more or less triangular spot; usual crescentic marks on the hind margin inconspicuous, sides of dorsum, including humeral calli, grey. Dorsum sparsely clothed with glistening yellow hairs; humeral calli with long whitish hairs. *Scutellum* chocolate-brown with hind margins and central region paler. *Pleurae* pale mouse-grey clothed with white hairs.

*Abdomen*: Dorsally rather darker than thorax with a relatively broad ashy-grey median stripe, and paired, more or less circular, similarly coloured, ill-defined, spots on the second to fifth segments; hind margins of segments, and part of front margin of second segment, narrowly ashy-grey, and extreme lateral margins of tergites one to four, light grey, the pale area becoming gradually smaller towards the fourth segment. Hairs clothing dorsum similar to those on thorax, but interspersed with black ones, especially on the darker areas and on the apical segment; lateral pale markings clothed with white hairs. *Venter* pale mouse-grey anteriorly and laterally, gradually merging into chocolate-brown towards the apex, hind margins with very narrow pale borders; clothed with short yellowish hairs proximally, with black hairs distally, and with hairs of either colour on the central segments.

*Wings*: Reddish-brown with rather coarse pale markings arranged as shown in Plate XIII, fig. 4. *Stigma*, elongate, dark reddish-brown, the basal third paler, yellowish-brown. *Squamae* whitish. *Halteres* with cream-coloured stems and dark brown knobs, the latter divided in the middle line by a fine creamy stripe.

*Legs*: Coxae dark grey; those of the fore legs clothed with short black hairs and some longer white ones towards the base, those of the middle and hind legs clothed with short whitish hairs. Femora dark brown, paler and greyish ventrally, clothed both with black and white hairs—the latter situated chiefly on the pale areas. Tibiae dark brown; front pair almost black, strongly incrassated and with a narrow cream-coloured band at the base; middle and hind pairs with two rather inconspicuous pale bands; tibiae, except

pale bands, clothed with black hairs, the bands with yellowish-white hairs. Front tarsi black, clothed with black hairs; middle and hind tarsi dark brown, the first segment of each mostly cream-coloured, and remaining segments of hind pair narrowly pale (cream-buff) at extreme base.

*Length*, 8.75-10 mm.; length of wing 7.4-8.7 mm.; width of head 2.6-2.9 mm.; width of front at vertex .9-1.05 mm.

*Habitat*: Onderstepoort, Transvaal; three females presented by Mr. F. V. Theobald (to whom I have much pleasure in dedicating this species), 1911, and one female by Mr. G. A. H. Bedford, 1913.

Mr. E. E. Austen, of the British Museum, has kindly examined one of the above specimens, and states that 'in some respects it appears to resemble *Haematopota bistrigata*, Loew, but is distinguishable at once from that species by *inter alia* the strongly incrassated front tibiae.'

*Haematopota pingucornis*, n. sp.

Small, dark, strikingly marked species; dorsal surface of thorax dark brown with a very broad, pale, median, longitudinal area on the anterior portion and a light coloured posterior margin; dorsum of abdomen dark brown with narrow greyish hind margins to the segments. Antennae dark brown, the first joint strongly incrassated and shining, the third joint short and flattened from side to side. Wings sepia with very conspicuous pale markings. Legs dark with pale bands on the tibiae, front pair with one pale ring, middle and hind pairs each with two pale rings—the hind tibiae somewhat dilated.

*Head* (fig. 5): *Face*, jowls and posterior region, grey, clothed with white hairs interspersed with a few short black ones; face with a conspicuous black transverse line extending from eye to eye, and passing immediately below the bases of the antennae, above this line the face, except the narrow whitish borders to the eyes, is yellowish-brown in colour becoming darker towards the antennae and margins. *Front* dull chocolate-brown with light grey lateral margins which extend inwards to enclose, or partially enclose, the lateral frontal spots and diagonally downwards, from the lateral corners of the vertex, to encircle the median frontal spot; vertex, between the two

diagonally projecting grey stripes, dark brown but with a fine grey median stripe extending to the median frontal spot. All three frontal spots distinct, the median spot rather small and circular, the lateral spots well-developed and more or less quadrate. Front somewhat denuded, apparently clothed with black and white hairs, the white hairs being limited to the paler areas. *Frontal callus* shining, dark brown or black, of moderate depth and with a distinct depression in the middle line; upper margin almost straight and narrowly separated from the eye at each end, lower margin slightly convex, its lateral extremities in contact with the eyes. Spot below callus well-defined, dark brown, quadrate. *Antennae* (fig. 5) dark, clothed on the hair-bearing portions with black hairs; first joint

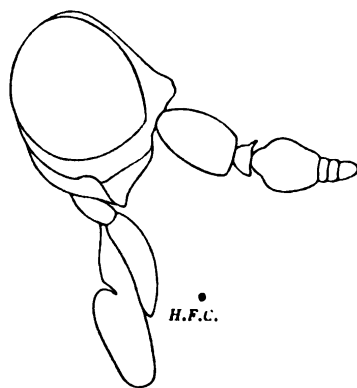


FIG. 5. *Haematopota pingucornis*, n.sp., side view of head, female. ( $\times 18$ , about).

much swollen, shining, dark brown but paler (yellowish-brown) towards the base and on the inner surfaces; third joint short, rather longer than the first and second joints taken together, with the expanded portion much compressed laterally and somewhat abruptly constricted basally; annuli fairly distinct, the last annulus about the same length as the two preceding together. *Palpi* grey; first segment darker and somewhat brownish, clothed chiefly with long white hairs, a few black ones present towards the apex; second segment rather narrow, clothed on the outer side with numerous relatively long black hairs, interspersed with a few white hairs.

*Thorax*: Pale median area on dorsum extending longitudinally from anterior margin of thorax to slightly beyond level of transverse

suture, its outer margins (corresponding to the usual admedian stripes) sharply defined, light grey, gradually darkening and becoming pale brownish in the central region, in which the median stripe may be traced as a narrow slightly paler line; light grey outer margins, immediately above inner lateral extremities of transverse suture, each curving inwards and uniting, near the middle line, with the inner edge of the light grey spot, situated below the inner lateral extremity of the transverse suture; crescentic marks on posterior margin large, light grey; humeral calli grey with white and black hairs intermingled, the white preponderating; sides each with an oval grey area, immediately behind humeral callus extending backwards as a moderately broad line to outer extremity of post-alar callus where, after a short interruption, it curves inwards and upwards forming a somewhat conspicuous spot. *Scutellum* dark brown, paler towards the upper margin. Dorsum clothed with both black and white hairs, their distribution corresponding more or less to the darker or lighter markings. *Pleurae* dark grey, clothed with white hairs.

*Abdomen*: Dorsum dark chocolate-brown, clothed with short black hairs, the hind and lateral margins of the tergites grey or yellowish-grey, clothed with white hairs. *Venter*, first segment greyish-brown, others with colouring and ornamentation similar to that on the tergites; clothed with short black hairs on the dark portions and glistening whitish hairs on the narrow grey margins, last ventral scute with numerous long black hairs.

*Wings* (Plate XIII, fig. 1): Dark with sharply defined coarse pale markings; apical sinuous mark strongly bifurcate, both branches broad, the outer ramus situated close to the wing margin; posterior cells, except fourth, and axillary cell with conspicuous pale blotches in their distal marginal angles, fourth posterior cell with a small indistinct pale mark in its distal angle; other markings as in the figure. *Stigma* somewhat elongate, very dark brown, conspicuous, with a dark rectangular blotch below extending nearly to upper margin of discal cell. *Squamae* white. *Halteres*, stalks cream-coloured, knobs dark brown.

*Legs*: Coxae dark brown, clothed with black hairs, basal half of front pair grey with long white hairs intermingled with the black ones. Femora dark brown, almost black, clothed with black hairs,

middle and hind femora rather paler ventrally and laterally, the hind pair slightly thickened. Tibiae, front pair black, each with a narrow cream-coloured band near the base, middle and hind pairs dark brown with two pale rings; whole of tibiae, except on the pale bands, clothed with black hairs, the pale bands with white hairs. Tarsi, front pair black, middle and hind pairs dark brown, each with the first segment mostly cream-coloured.

*Length*, 6.3 mm.; length of wing 6.8 mm.; width of head 2.5 mm.; width of front at vertex .8 mm.

*Habitat*: Lorha, Gold Coast; Dr. J. F. Corson, 18.4.1914; one female.

This prettily marked species does not seem to be closely related to any West African form.

*Haematopota angustifrons*, n. sp.

Dark medium-sized species with a relatively narrow front; dorsum of thorax dark sepia-coloured, with two pale admedian stripes on the anterior half, light grey posterior border and pale scutellum; dorsal surface of abdomen dark with traces of paired admedian spots on the apical segments. Wings warm sepia-coloured with coarse creamy-yellow markings. Legs dark, tibiae with the usual pale bands, front tibiae slightly dilated, hind tibiae uniformly thickened.

*Head* (fig. 6): *Face*, jowls and basioccipital region light grey clothed with glistening yellowish hairs; face with two circular black spots, one immediately below the base of each antenna. *Front* narrow, its length about one and five-eighths times the width at the vertex, dark velvety brown in colour with very narrow yellowish-brown lateral margins extending to encircle the frontal spots; vertex with a darker brown, more or less triangular, blotch divided in the middle line by a fine pale stripe; front clothed with short black hairs, sparse golden-brown hairs on the paler areas. Three frontal spots present, the median small but distinct, the laterals large, somewhat rhomboidal in shape, and either narrowly separated from or in contact with the eyes. *Frontal callus* deep, shining, almost black, not extending from eye to eye, prominent; upper margin rounded but slightly depressed on each side of the middle line, lower margin concave above the antennae. Dark spot below callus,

quadrate, conspicuous. *Antennae* (fig. 6) first joint dark yellow becoming yellowish-grey towards the apex, long and cylindrical, not incrassate, clothed with black hairs; second joint similarly coloured, its upper angle very slightly produced; third joint elongate, dark brown to dark yellowish-brown, paler, yellowish-brown at extreme base; annuli distinct, almost black, the third very

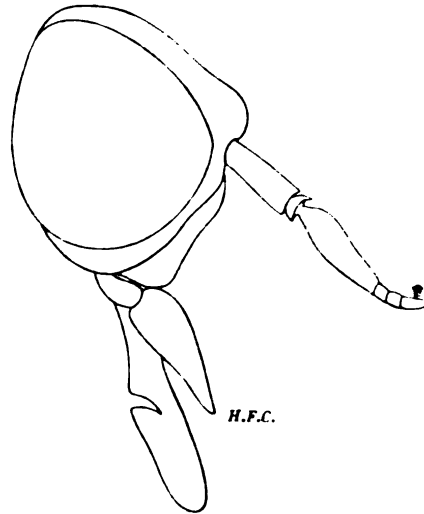


FIG. 6. *Haematopota angustifrons*, n.sp., side view of head, female. ( $\times 18$ , about).

little shorter than the two preceding taken together. *Palpi* first segment greyish-yellow, clothed with long glistening cream-coloured hairs ventrally and laterally, and sparsely with long black hairs dorsally; second segment, except a large area on the outer side, greyish-yellow, clothed with long cream-coloured hairs, outer side mostly dark grey covered with short black hairs.

*Thorax*: Admedian stripes on dorsum, creamy white, extending nearly to the inner extremities of the transverse suture, each followed immediately below suture by a conspicuous, triangular, similarly coloured, spot; crescentic marks on posterior margin, creamy-white, sharply defined and relatively large; dorsum clothed with golden-yellow and minute black hairs intermixed, those of the former colour more numerous on the sides of the anterior region and longer and denser on the posterior region; humeral calli mouse-grey clothed with long whitish hairs. *Scutellum* creamy-white with dark hind

border, the pale area prolonged in the middle line in some specimens to the apex; clothed with creamy-white hairs. *Pleurae* greyish-brown above, slate-grey below, clothed with pale yellowish hairs.

*Abdomen*: Slightly darker than the thorax with yellowish hind margins to the segments and a pair of ill-defined, elongated, dull yellowish, admedian spots on the fourth, fifth and sixth tergites; clothed with short black hairs on the main portion, yellowish hairs on the paler hind margins and rather long, dense, glistening white or yellowish-white hairs on the extreme lateral apical corners. *Venter* greyish-brown darkening towards the apex, with hind margins yellow but becoming greyish laterally; clothed with glistening yellowish hairs interspersed with black hairs in the central region of the penultimate segment, and with erect black hairs on the last segment.

*Wings* (Plate XIII, fig. 2): Pale markings coarse, creamy-yellow and conspicuous; rosettes large and distinct; apical sinuous mark relatively broad in its upper and lower portions but greatly narrowed and sometimes interrupted near the middle of the apical cell; posterior cells, except the fourth, with very large pale blotches along the wing margin, that in the first being formed by the lower extremity of the apical mark; axillary cell with a broad clearly-defined pale band running from the basal loop round the proximal margin and joining a somewhat sinuous pale line which extends upwards to the fifth longitudinal vein, and with a large pale blotch in its distal angle which extends well into the proximal angle of the fifth posterior cell. *Stigma* elongate, conspicuous, brownish-black, the extreme basal portion yellowish; veins brownish-black. *Squamae* brown. *Halteres* entirely pale cream-coloured.

*Legs*: Front pair black, middle and hind pairs dark brown. Coxae of the first pair of legs dark brown or black with the proximal fourth greyish; clothed with long white hairs basally and with black hairs on the remaining portion. Femora, front pair clothed with black hairs which are rather long on the dorsal surface; middle and hind pairs clothed with black and occasional white hairs, those on the ventral surface of the hind pair being somewhat longer and more noticeable than usual. Front tibia with a broad white basal band, middle and hind tibiae each with two yellowish bands, the distal bands on the hind pair being generally incomplete, narrower and

darker; greater part of tibiae clothed with dark hairs, pale bands with white or yellowish hairs, interspersed on the hind pair with black hairs—dorsal surface of hind tibiae with rather long dense black hairs. Tarsi of middle and hind legs with the first segments narrowly cream-coloured at the base; clothed with black hairs.

*Length*, 8.8-10 mm.; length of wing 8.5-9 mm.; width of head 3.25-3.5 mm.; width of front at vertex .65-.75 mm.

*Habitat*: Belgian Congo; Dr. E. Dutton and Dr. J. L. Todd, 1904; six females.

The exact position of *H. angustifrons* is somewhat difficult to define. In a general way it resembles *H. grahami*, Aust., and displays affinities to this species in the narrowness of the front, the shape of the frontal callus and, to a certain extent, in the arrangement of the pale markings on the body. The most striking character of *H. grahami*, however, namely the presence of fringes of long hair on the femora and hind tibiae, is not present, although the hairs on these parts in *H. angustifrons* are perhaps more conspicuous than usual.

*Haematopota exiguicornuta*, n. sp.

Small obscurely marked species with extremely short antennae; dorsal surface of thorax dull brown with three ill-defined, pale, longitudinal stripes; dorsum of abdomen slightly darker than that of the thorax, with inconspicuous admedian paired spots on some of the segments. Legs somewhat pale, the tibiae ringed. Wings with rather coarse pale markings and inconspicuous stigma.

*Head* (fig. 7): *Face*, jowls and basioccipital region light grey, clothed with white hairs; upper part of face, at the side of each antenna, dark brown with a narrow black lower border and with yellowish pubescence. *Front* sepia-coloured, slightly paler round the lateral frontal spots and along the margins of the eyes; clothed with short dark brown hairs. All the frontal spots present but the median spot much reduced and scarcely visible under the pocket lens, lateral frontal spots separated from the eye on each side, moderately conspicuous, and more or less diamond-shaped, each with its longer axis pointing inwards and downwards. *Frontal callus* in the form of a narrow transverse dark reddish-brown band extending nearly from eye to eye; upper and lower margins gently curved and almost parallel, the former slightly concave. Spot



below callus small dark brown and divided in the middle line. *Antennae* (fig. 7) pale, shorter than the head; first joint short, dark reddish-brown, partly shining and swollen, clothed with black hairs; second joint paler brown, its upper angle considerably produced; third joint very short, yellowish, considerably flattened from side to side, the basal portion almost as broad as long; annuli (at least the first and second) indistinctly separated, the last not as long as the two preceding taken together and darker brown

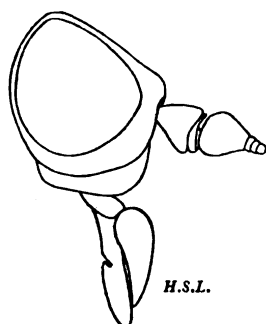


FIG. 7. *Haematopota exiguicornuta*, n.sp., side view of head, female. ( $\times 18$ , about).

at apex. *Palpi* mouse-grey; first segment clothed with long white hairs, except dorsally and apically, where black hairs occur; second segment somewhat dilated basally and gradually tapering to a bluntly rounded apex, clothed with long glistening white hairs on the ventral surface, and on the outer side with shorter black hairs.

*Thorax*: Longitudinal greyish stripes on dorsal surface inconspicuous, the median stripe extending nearly to the scutellum, the admedian stripes reaching almost to the transverse suture, each with an indication of the usual spot below its posterior extremity; crescentic marks on posterior margin faintly marked, hardly recognisable; sides slightly paler; dorsum sparsely clothed with short glistening white and black hairs intermixed. *Scutellum* dull brown with greyish hind margin, clothed with hairs similar to those on the main portion of the dorsum. *Pleurae* grey, clothed with glistening whitish hairs.

*Abdomen*: Hind and lateral margins of tergites narrowly light grey, the pale area being comparatively broad on the sides of the first and second segments; ill-defined admedian spots present on the second to seventh tergites inclusive; dorsum clothed mainly

with black hairs, pale margins with white hairs. *Venter* similar to dorsum, but without admedian spots, with the first segment grey and with the white hairs more widely and irregularly distributed.

*Wings*: Pale sepia, the light markings somewhat indistinct and arranged as shown in Plate XIII, fig. 5. *Stigma* very faintly marked, the distal two-thirds pale brown, the proximal third whitish; veins reddish-brown. *Squamae* sepia-coloured. *Halteres*, stems cream-coloured, knobs dark brown with a relatively broad creamy stripe along the middle line.

*Legs*: Pale brown. Coxae grey, clothed on the apex with black hairs and on the remaining portion with white hairs. Femora mostly greyish pollinose, clothed with black hairs, interspersed, especially ventrally and laterally, with white hairs. Tibiae pale brown, the front pair with a pale basal ring, the middle and hind pairs each with two pale rings; clothed chiefly with black hairs, sparse white hairs (which are more numerous on the hind tibiae) being present on the pale rings. Tarsi, front pair dark brown, middle pair yellowish-brown, hind pair rather paler than front pair, each with the base of the first segment yellowish; all clothed with black hairs.

*Length*, 6 mm.; length of wing, 6.2 mm.; width of head, 2.5 mm.; width of front at vertex, 0.7 mm.

*Habitat*: Parade ground, Lokoja, N. Nigeria; Dr. W. M. Manuk, 19.7.1910; one female.

The peculiar and characteristic antennae of *H. exiguicornuta* render its identification, and separation from species which resemble it in general facies, a comparatively simple matter.

*Haematopota corsoni*, n. sp.

Small dull sepia-coloured species with short antennae and relatively large dark areas on the face; dorsum of abdomen with indications of paired spots and with narrow pale hind margins to the segments. Wings grey, the pale markings somewhat reduced and inconspicuous; stigma well marked. Legs pale brown, tibiae unbanded.

*Head* (fig. 8): *Face* light grey with a conspicuous black area extending between the antenna and the eye on each side, narrowly separated at its extremities from both, and with the greater part of the central portion, below the antennae, dark-brown; jowls and

basioccipital region light grey, clothed with long whitish hairs. *Front*, sepia with narrow yellowish-grey lateral margins extending partly round the lateral frontal spots. Median frontal spot absent or nearly so, lateral spots more or less quadrate and in contact with the eyes. *Frontal callus* narrow, shining, reddish-brown, almost divided by a longitudinal depression in the middle line and with the lateral extremities of its lower margin touching the eyes; upper margin nearly straight, curving downwards for a short distance in the centre, lower margin slightly concave above the base of each antenna. Spot below callus well-defined, dark brown, triangular, its apex directed downwards. *Antennae* (fig. 8) dark yellowish-brown, the

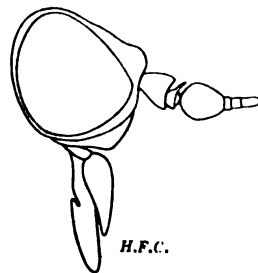


FIG. 8. *Haematopota corsoni*, n.sp., side view of head, female. ( $\times 18$ , about).

tips (annuli) dark brown; first joint slightly incrassate and shining, clothed with short black hairs; second joint with the upper angle moderately produced; expanded portion of third joint short—slightly longer than broad—and somewhat flattened from side to side, terminal annuli taken together as long, or almost as long, as the expanded portion, last annulus equal in length to the first and second combined. *Palpi* grey, the outer side of the second segment with short black hairs.

*Thorax*: Dorsum dull sepia, with three inconspicuous, smoke-grey, longitudinal stripes extending from the anterior margin to the region of the transverse suture, the spots below the posterior extremities of the admedian stripes very faintly marked; crescentic marks absent; sides, except a small area immediately behind each humeral callus, and *scutellum* dark; humeral calli and areas indicated grey, the former clothed with long white hairs; dorsal surface sparsely clothed with short whitish or yellowish-white hairs. *Pleurae* grey, clothed with white hairs.

*Abdomen*: Dorsum slightly darker than the thorax, the tergites with very narrow brownish-grey hind margins, that on the second expanding into a small greyish triangle in the middle line; lateral margins of first to fifth segments greyish; paired, admedian spots on the second to sixth tergites, indistinct, brownish-grey in colour and approximated to the anterior margins. *Venter* generally rather darker than the dorsum, but with the whole of the first and second segments, except the central portion of the latter, mouse-grey; hind and lateral margins paler, the hind margins of the proximal scutes light grey. Both surfaces of abdomen clothed with white and black hairs, the white hairs limited to the pale margins of the tergites, but more widely and irregularly distributed on the sternites.

*Wings* (Plate XIII, fig. 6): Pale markings white, inconspicuous, distributed as shown in the illustration. *Stigma* sepia, the extreme base pale. *Squamae* white, becoming yellowish towards the margins. *Halteres*, stems mostly cream-coloured, but darker at the base, knobs dark brown with a yellowish-brown central stripe.

*Legs*: Coxae steel-grey, clothed with glistening white hairs. Femora, front and hind pairs dull pale-brown, middle pair somewhat yellowish-brown; clothed with short black and white hairs intermingled. Tibiae similar in colour to the femora, without the usual pale rings but with a few short white hairs present on those positions where the pale bands generally occur, thereby giving the tibiae faint indications of bands. Tarsi, front pair slightly darker than the middle and hind pairs, the first segment of each of the middle and hind pairs faintly yellowish or yellowish-brown at the base.

*Length*, 5.4 mm.; length of wing, 5.5 mm.; width of head, 2 mm.; width of front at vertex, 0.6 mm.

*Habitat*: Salaga (15 miles North), Gold Coast; Dr. J. F. Corson (per Mrs. J. F. Corson), 17.10.1914; one female.

This small and obscure species may, on a casual examination, easily be confused with *Haematopota exiguicornuta*, n. sp. It may be readily distinguished, however, by the less swollen first antennal segment and the longer and more distinctly separated terminal annuli of the third joint, by the more conspicuous stigma and different arrangement of the pale markings on the wings, and by the unbanded tibiae, as well as by certain other, less obvious, differences.

WEST AFRICAN SPECIES OF *HAEMATOPOTA*

Since three of the species of *Haematopota* described above were obtained in West Africa, a tabular statement of the more important distinctive characters of those members of the genus recorded from the British Possessions on this coast may prove of some value to medical officers and others interested in the biting flies of this region. The following table, therefore, refers to the *females* of those species at present known to occur in Gambia, Sierra Leone, Gold Coast or Nigeria, and its preparation has been greatly facilitated by Mr. E. E. Austen's excellent descriptions of his numerous species.

- |  |                              |
|--|------------------------------|
| 1. Front and hind femora and tibiae fringed with long coarse hair ...  | 2                            |
| Front and hind femora and tibiae not fringed with long coarse hair ...   | 3                            |
| 2. Hind tibiae greatly swollen with two ill-defined pale rings; frontal callus widely separated from the eyes; grey thoracic markings extensive ...                                      | <i>bullatifrons</i> , Aust.  |
| Hind tibiae scarcely swollen with one broad pale ring; frontal callus almost touching the eyes; grey thoracic markings not extensive ...   | <i>grahami</i> , Aust.       |
| 3. Legs ornate with pale rings on the tibiae (distinct on, at least, the front pair) ...   | 4                            |
| Legs inornate or with obscure rings on the tibiae ...  | 12                           |
| 4. Hind tibiae with one pale ring ...  | 5                            |
| Hind tibiae with two pale rings ...  | 6                            |
| 5. Blackish species with extensive grey thoracic markings, conspicuously banded tibiae and pronounced milky-white wing markings  | <i>decora</i> , Walk.        |
| Dusky species with dark wings, narrow grey thoracic markings and less distinctly banded tibiae ...   | <i>bastata</i> , Aust.       |
| 6. Anterior tibiae with two pale rings (the distal ring often faint) ...   | 7                            |
| Anterior tibiae with one pale ring ...   | 8                            |
| 7. Larger (10-12 mm.) reddish-brown species; first antennal segment distinctly swollen; abdomen with a rather broad median stripe, and with traces of spots on the terminal segments ... | <i>vittata</i> , Loew.       |
| Smaller (9.6 mm.) sepia-coloured species; first antennal segment not swollen; abdomen with a narrow median stripe and large spots on all segments ...                                    | <i>puniens</i> , Aust.       |
| 8. Antennae longer than the head ...   | 9                            |
| Antennae very short and inconspicuous, shorter than the head (basal portion of third joint greatly expanded) ...   | <i>exiguicornuta</i> , n.sp. |

9. First joint of antenna not distinctly swollen ... .. 10  
     First joint of antenna distinctly swollen and shining, small dark species  
     with conspicuously-marked wings ... .. *pinguicornis*, n.sp.
10. Brown species; background of wings dark, the light markings  
     distinct ... .. 11  
     Greyish species; background of wings very pale, the light markings  
     indistinct ... .. *pallidipennis*, Aust.
11. Scutellum brown; first joint of hind tarsi partly yellowish.  
     *torquens*, Aust.  
     Scutellum grey; first joint of hind tarsi entirely brown.  
     *cordigera*, Bigot.
12. Brownish-yellow or greyish species with conspicuous stripes on the  
     thorax ... .. 13  
     Dark brown species without conspicuous stripes on the thorax (but cf.  
     *tenuicrus*, Aust.) ... .. 15
13. Central part of wing hyaline or semi-hyaline, only the tips and hind  
     border infuscated and with inconspicuous pale markings ... 14  
     Wings sepia with clearly defined pale markings, extending to the base  
     *pertinens*, Aust.
14. Brownish-yellow species; frontal callus yellowish ... *beringeri*, Aust.  
     Greyish species; frontal callus dark brown ... *semiclara*, Aust.
15. Anterior tibiae not dilated ... .. 16  
     Anterior tibiae distinctly dilated; small dark species with first joint of  
     antenna swollen ... .. *laccessens*, Aust.
16. Antennae moderately elongate; basal portion of third joint slender 17  
     Antennae very short, scarcely longer than the head; basal portion of  
     third joint broad ... .. *corsoni*, n.sp.
17. Thorax conspicuously striped, abdomen indistinctly spotted; first  
     antennal segment very short and somewhat swollen...*tenuicrus*, Aust.  
     Thorax inconspicuously striped, abdomen unspotted; first antennal  
     segment longer and not swollen ... .. *gracilis*, Aust.

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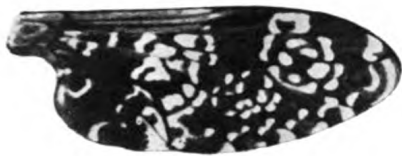
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## EXPLANATION OF PLATE XIII

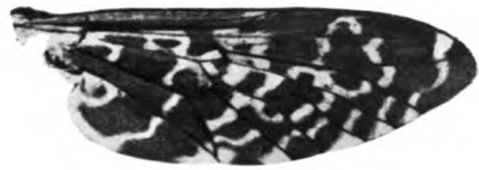
- Fig. 1. Wing of *Haematopota pinguicornis*, n. sp., female ( $\times 7\frac{1}{2}$ ).  
Fig. 2. Wing of *Haematopota angustifrons*, n. sp., female ( $\times 7$ ).  
Fig. 3. *Haematopota transvaalensis*, n. sp., female ( $\times 3$ ).  
Fig. 4. *Haematopota theobaldi*, n. sp., female ( $\times 3$ ).  
Fig. 5. Wing of *Haematopota exiguicornuta*, n.sp., female ( $\times 7\frac{1}{2}$ ).  
Fig. 6. Wing of *Haematopota corsoni*, n. sp., female ( $\times 7\frac{1}{2}$ ).

NOTE.—In figs. 1, 2, 5 and 6 the background of the wings has been darkened in order to render the light markings more conspicuous and to overcome halation.





I



2



3



4



5



6



## EXPERIMENTS WITH SALVARSAN-COPPER IN TRYPANOSOMIASIS

BY

HARALD SEIDELIN, M.D.

*(Received for publication, 23 February, 1915)*

WITH TABLE

Just before I left for West Africa, in July, 1913, I received from Geheimrat Ehrlich a supply of a new preparation, K<sub>3</sub>, salvarsan-copper, which he asked me to use in the treatment of yellow fever cases. Unfortunately I had no opportunity of complying with this request, because I saw only yellow fever cases of a mild type, in which an indifferent treatment was obviously all that was required. Several severe cases occurred during my stay in Lagos, but they were either convalescent when they came under my observation, or they died almost immediately after admission to hospital.

It was also my intention to try the treatment in experimental yellow fever infection in guinea-pigs, but here again the conditions were unfavourable. The severity of the experimental infection varied considerably, and it was never possible to tell whether an animal would survive or succumb to the disease. Experiments of this nature would have to be conducted with large parallel series of infected and non-infected animals, and such experiments we were not in a position to undertake.

Under these circumstances I resolved to test the value of K<sub>3</sub> in trypanosome-infections. For this purpose, Dr. J. W. Scott Macfie kindly supplied me with a strain of a trypanosome (*T. brucei* group) which he had for some time kept in guinea-pigs and rats.

The results of my experiments are given in the accompanying table, which requires but little explanation. The animals experimented upon were white rats; the infection was transmitted by means of intraperitoneal injections of a few drops of blood drawn from the cavernous sinus, according to the technique described by Pettit (1913). The trypanosome strain proved very virulent, the infected animals, when not treated, dying from eight to seventeen days after inoculation.

Geheimrat Ehrlich had advised me to start with doses of 0.05 or

0.10 gramme in experiments upon human patients. The average weight of the rats was about 60 grammes, and the variations in weight were small; thus, the proportion in weight of rat to man would be approximately 1 : 1,000. Accordingly, in the first experiment I took 0.0001 as a medium dose, using in addition two higher and two lower doses. As no satisfactory result was obtained in this experiment I increased the dosage, always proceeding by multiples of 2. It will be seen from the table that no striking result was obtained until I reached a dose of eight times the one recommended, and that the best results, as far as the prolonged absence of the parasites from the blood was concerned, were obtained by the injection of 0.0064 gramme instead of 0.0001 gramme. In such a case the trypanosomes, which had been very numerous before the injection, were absent from the blood on the following day and remained absent for fifteen days; the animal died twenty-eight days after the inoculation. In several other cases I succeeded in prolonging the life of the animals for a few days more, although the parasite-free interval was of shorter duration. It appears probable that repeated injections of smaller quantities would have given better results, but this method of treatment was not fully investigated. The number of animals available was not sufficient for work on different lines, and I therefore confined myself to the investigation, in the first instance, of what I regarded as the problem of primary importance, namely, the possibility of establishing a real '*therapia sterilisans magna*.' In this I failed: a single injection, however large, did not kill all the parasites in an infected animal, though it made them disappear, for a considerable length of time, from the peripheral blood. It would hardly be possible, even in experiments on animals, to use larger doses than those I employed, as several animals died a comparatively short time after the injection without reappearance of the parasites, and in others a more or less extensive necrosis of the tissues was produced at the site of injection.

The results may be discussed in a few words.

In series A very little effect was obtained. No appreciable numerical reduction of the parasites was observed, even after the injection of 0.0004 gramme  $K_3$ , but in all cases the parasites appeared much less lively on the day following the injection than they had been the day before and as compared with the parasites in

the blood of the control animals on the same day. The longest period of survival obtained in the two animals which had received the largest doses of  $K_3$ .

In series B the action of the drug was evident. Two animals survived the inoculation for 28 and 29 days, respectively, and in one of them trypanosomes were absent from the blood for six days; this animal had received an injection of 0.0016 gramme  $K_3$ .

In the two previous series only the doses administered had been varied, but in series C the drug was injected into animals which were in different stages of infection. In three animals, as in all those belonging to the series A and B, trypanosomes were numerous at the time of injection, but in the other three they had not yet appeared in the peripheral blood; one animal of the latter group had been inoculated about 75 minutes before the therapeutical injection. The stage of infection proved to be of little importance. The hope had been entertained that a dose, which showed a marked effect when numerous trypanosomes were present in the blood, might entirely destroy an infection in its initial stage; but this hope was disappointed. In rat 232, trypanosomes did not appear in the peripheral blood until eight days after inoculation; this free interval may be assumed to be composed of two periods, namely, one corresponding to the action of  $K_3$ , followed by another corresponding to the stage of incubation. The dose administered was 0.0016 gramme. The same dose was given in the cases 199, 220, and 229, in which cases the parasite-free intervals varied from four to six days. Thus, if we reckon five days for the first period in rat 232, we get three days for the second period, which is precisely the usual duration of the incubation stage. In the case of rat 221, trypanosomes reappeared in the peripheral blood on the tenth day after the injection of 0.0032 gramme  $K_3$ ; on the same day 0.0064 gramme was injected, whereupon the trypanosomes again disappeared from the blood and remained absent until the animal died, twenty-three days after inoculation.

In series D the doses were increased. The parasite-free intervals were prolonged, the maximum of fifteen days after a single injection being attained in the case of rat 241, which received 0.0064 gramme  $K_3$ ; but the animals did not survive as long as those in experiment C.

In series E repeated injections were given, the dose in each instance being 0.0064 gramme; in this way a parasite-free period of twenty days was obtained, but the length of survival was not increased.

In several of the experiments of the series D and E the animals died without the trypanosomes having reappeared in the blood.

The experiments in series F were undertaken in order to test the possibility of producing a  $K_3$ -resistant strain of the trypanosome. Two rats were inoculated with blood from an animal in which the parasites had first disappeared after the injection of  $K_3$ , and afterwards reappeared. One of the animals was injected twice with  $K_3$ , and the effect of the drug was considerably less than in the other experiments.

The results here reported are interesting from a general point of view, and leave no possible doubt as to the powerful action of salvarsan-copper upon this strain of trypanosome, but they are not conclusive with regard to the therapeutical value of the drug. After having concluded my experiments, I became acquainted with the paper by Van den Branden (1913) on the effect of  $K_3$  in human trypanosomiasis. His results are far superior to those obtained by me in rats and with a different species of *Trypanosoma*; this confirms the information given me by Professor Ehrlich that  $K_3$  has a markedly different effect upon different trypanosomes.

The experiments here recorded were carried out whilst I was working for the Yellow Fever (West Africa) Commission of the Colonial Office, at the Medical Research Institute, at Yaba, near Lagos, Nigeria. To the Director of the Institute, Dr. A. Connal, I am indebted for the facilities afforded me.

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Rat No.	Date inoculation 191					Result		
	16	17	18	19	20			
161	22.2	---	...	...	...	Death 13 days after inoculation	Spleen very much enlarged	
162	22.2	---	...	...	...	" 7 " "	Peritoneal infection	
163	22.2	---	...	...	...	" 14 " "		
164	22.2	---	...	...	...	" 8 " "	Spleen very much enlarged	
169	22.2	---	...	...	...	" 11 " "	" "	
165	22.2	---	...	...	...	" 13 " "	" "	
166	22.2	---	...	...	...	" 14 " "	" "	
167	22.2	---	...	...	...	" 17 " "	" "	
168	22.2	---	...	...	...	" 17 " "	" "	
159	22.2	---	...	...	...	" 7 " "	" "	
160	22.2	---	...	...	...	" 14 " "	" "	

195	15.2	...	...	...	...	Death 12 days after inoculation		
196	15.2	...	...	...	...	" 13 " "		
197	15.2	...	...	...	...	" 13 " "		
198	15.2	+++	+++	...	...	" 29 " "		
199	15.2	+++	+++	...	...	" 28 " "		
194	15.2	...	...	...	...	" 15 " "		

220	28.	...	...	...	...	Death 28 days after inoculation		
221	28.	—	...	...	...	" 23 " "		
229	L	...	...	...	...	" 22 " "		
230	4	...	...	...	...	" 32 " "		
231	3	...	...	...	...	" 33 " "		
232	4	...	...	...	...	" 22 " "		

[P.T.O.]

In series E repeated injections were given, the dose in each instance being 0.0064 gramme; in this way a parasite-free period of twenty days was obtained, but the length of survival was not increased.

In several of the experiments of the series D and E the animals died without the trypanosomes having reappeared in the blood.

The experiments in series F were undertaken in order to test the possibility of producing a  $K_3$ -resistant strain of the trypanosome. Two rats were inoculated with blood from an animal in which the parasites had first disappeared after the injection of  $K_3$ , and afterwards reappeared. One of the animals was injected twice with  $K_3$ , and the effect of the drug was considerably less than in the other experiments.

The results here reported are interesting from a general point of view, and leave no possible doubt as to the powerful action of salvarsan-copper upon this strain of trypanosome, but they are not conclusive with regard to the therapeutical value of the drug. After having concluded my experiments, I became acquainted with the paper by Van den Branden (1913) on the effect of  $K_3$  in human trypanosomiasis. His results are far superior to those obtained by me in rats and with a different species of *Trypanosoma*; this confirms the information given me by Professor Ehrlich that  $K_3$  has a markedly different effect upon different trypanosomes.

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- PETIT, A. (1913). Procédé simple pour prélever du sang chez les petits rongeurs. *C. R. Soc. Biol.*, Paris, 10 jan., LXXIV, 1, pp. 11-12.



Rat No.	Date of inoculation 1913					Result		
	16	17	18	19	20			
161	22.X	...	...	...	...	Death 13 days after inoculation		
162	22.X	...	...	...	...	" 7	"	Spleen very much enlarged
163	22.X	...	...	...	...	" 14	"	Peritoneal infection
164	22.X	...	...	...	...	" 8	"	Spleen very much enlarged
169	22.X	...	...	...	...	" 11	"	" "
165	22.X	...	...	...	...	" 13	"	" "
166	22.X	...	...	...	...	" 14	"	" "
167	22.X	...	...	...	...	" 17	"	" "
168	22.X	...	...	...	...	" 17	"	" "
159	22.X	...	...	...	...	" 7	"	" "
160	22.X	...	...	...	...	" 14	"	" "

195	15.XI	...	...	...	...	Death 12 days after inoculation		
196	15.XI	...	...	...	...	" 13	"	"
197	15.XI	...	...	...	...	" 13	"	"
198	15.XI	+++	+++	...	...	" 29	"	"
199	15.XI	+++	+++	...	...	" 28	"	"
194	15.XI	...	...	...	...	" 15	"	"

220	28.XI	...	...	...	...	Death 28 days after inoculation		
221	28.XI	-	...	...	...	" 23	"	"
229	1.XI	...	...	...	...	" 22	"	"
230	2.XI	...	...	...	...	" 32	"	"
231	3.XI	...	...	...	...	" 33	"	"
232	4.XI	...	...	...	...	" 22	"	"

[P.T.O.]

240	8.XII	13.XII	•	...	...	...	Death 11 days after inoculation	
241	8.XII	13.XII	•	...	...	...	„ 28 „ „	Necrosis at the site of injection
243	10.XII	...	•	...	...	...	„ 17 „ „	Large necrotic cavity at the site of injection
244	10.XII	13.XII	•	...	...	...	„ 3 „ „	
247	11.XII	13.XII	•	...	...	...	„ 4 „ „	
250	12.XII	13.XII	•	...	...	...	„ 10 „ „	Spleen very slightly enlarged

289	30.XII	4.I.14	•	...	...	...	Death 19 days after inoculation	
290	30.XII	4.I.14	•	—	—	— inj. of 0.00064 g. K <sub>8</sub>	„ 26 „ „	

appeared; subsequent treatment with K<sub>8</sub>.)

227	1.XII	4.XII	• +	+++	+++	+++	Death 25 days after inoculation	
228	1.XII	...	•	...	...	...	„ 13 „ „	



# STUDIES IN BLACKWATER FEVER

## III.—THE RELATIONSHIP OF QUININE TO BLACKWATER FEVER\*

BY

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AND

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*(Received for publication 24 February, 1915)*

In the literature of blackwater fever contradictory statements are made concerning the rôle of quinine, some observers asserting that quinine induces the attack of blackwater, others denying this. The solution of the question is not advanced by the affirmation or denial of supposed authorities. What we have tried to find out is whether a statistical examination of the times at which quinine is given and blackwater occurs respectively, reveals any relationship between the two.

In examining the literature, an initial difficulty confronted us, viz., the incompleteness of the records. For our purpose it was, as will be seen, necessary to know as exactly as possible at what hour quinine was administered and at what hour the blackwater ensued. It was often impossible to ascertain these facts, and we would state clearly that the only data we have used are those in which the exact times of the giving of quinine and the occurrence of the rigor or blackwater are stated.

We have thus not considered cases where the statements are not more definite than, e.g., 'quinine was given the day before,' 'the day of,' 'shortly before the blackwater,' etc., etc., and also, of course, those cases could not be considered where it was stated that *no* quinine had been taken.

As it was practically impossible to get records of cases with

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\* Part I. *Annals of Trop. Med. and Parasit.* (1913). Vol. VII, p. 479.  
Part II. *Ibid.* (1914). Vol. VIII, p. 639.

a complete quinine history we were not able to study such, consequently we proceeded to examine whether any relationship exists between the time of the blackwater and the time of the *last dose* of quinine.

In some cases the time of the rigor is stated, in others the time of the blackwater. Very probably the time of the onset of the rigor marks the real onset of the attack and is perhaps more accurate in that respect than the time of the passage of blackwater, for we have no accurate knowledge as to how long haemoglobinous urine can be retained, although it seems to be generally assumed that haemoglobinous urine is irritating to the bladder.

The data then that concern us are (1) the time when the last dose of quinine was given; (2) the time of onset of the rigor; (3) the time of first passage of blackwater.

We have obtained these or some of these data in 372\* cases, the records of which we have examined.

*Time of quinine:* This was given in 157 out of 372 cases.

*Time of rigor:* This was given in 121 out of 372 cases.

*Time of blackwater:* This was given in 217 out of 372 cases.

It will be noted that it is possible either (1) that the 157 cases in which the time of quinine is given are none of them the same cases as the 121 cases in which the time of the rigor is given, or (2) that the 157 cases (except 2) of quinine are none of them the same as the 217 cases in which the time of the blackwater is given, but although both these possibilities could not hold good for all the cases in categories (1) and (2), they might do so for a part of both, so that in the first table the fields of observation are not (entirely) coincident. At present we disregard this.

The data were now arranged as in Table I. We have taken eight three-hour periods, because it was (frequently) impossible to define the time more accurately, and also because if the day were divided up into twenty-four hours the number of cases falling under each hour would be too small whereon to base any deductions.

On examining the columns of deviation from an average, it will be noticed that each has a very decided maximum.

(1) *Time of taking quinine.* Upon the assumption that a random

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\* Four cases have been excluded in which the interval between the quinine and the onset of the rigor or blackwater was greater than 24 hours.

distribution\* is *a priori* to be expected, it can be calculated that the large deviation, 28 in excess of the average, would only be expected to occur once in *over* 10,000 cases, so we may fairly say that the distribution is not a random one, and that there is some reason for taking quinine between the hours of 6 a.m. and 9 a.m.

This we can assume is due to our methods of life, the time of rising in the morning determining the time of taking quinine.

TABLE I

Hour	Number of people taking quinine at stated hour	Deviations from average of 20 per 3 hours	Number of rigors occurring at stated hour	Deviations from average of 15 per 3 hours	Number of cases of blackwater at stated hour	Deviations from average of 28 per 3 hours	
A.M.	12—3	3	—17	8	—7	18	—10
	3—6	18	—2	7	—8	15	—13
	6—9	48	+28	14	—1	20	—8
	12—6	26	+6	38	+23	47	+19
P.M.	12—3	12	—8	22	+7	50	+22
	3—6	22	+2	10	—5	37	+9
	6—9	16	—4	9	—6	17	—11
	9—12	12	—8	13	—2	13	—15
	157		121		217		

(2) *The onset of rigor.* The maximum deviation in this case is between the hours of 9 a.m. and 12 noon. Here we have 23 above the average, and it is also at least 10,000 to one that this does not occur at random, that is, there is some cause at work which determines the time.

(3) *Passing of blackwater.* Here we have the maximum, 22,

\* By a random distribution in this case, we mean the distribution that would be expected to occur, if each case was entered at random in any of the eight divisions into which the day was divided, by a person who had no bias in favour of one hour rather than another, or if the division into which each case was entered was determined by picking a ball out of a bag containing eight differently coloured balls. Out of 157 cases we should expect *about* 20 in each of the eight divisions, but almost certainly there would be slight departures, owing to our random method of filling the divisions, but we have in our 6-9 a.m. division as many as 48 that is a departure from the average, 20, of 140 per cent.

between 12 and 3 p.m., but there is also a large positive deviation, 19, between 9 a.m. and 12 noon. This would be expected to happen if the period between quinine and blackwater were a little over 3 hours, many cases falling on the border line at noon. Again such a large deviation as 22 would only occur by chance once in 10,000 cases.

If now we move the figures for the rigor 3 hours back, the reason for so doing we shall presently see, and compare them with those of the quinine, we find that the coefficient of correlation ( $r$ ) between them is  $r = .92 \pm .04$ , a very strong correlation ( $r = 1$  is perfect correlation). In non-mathematical language, correlation, positive or negative, means 'fit,' and we may illustrate firstly the meaning of *positive* correlation in the following way:—

Take sheets of paper in pairs, subject each pair to some operation, e.g., cutting with a pair of scissors through both at the same time; if we cut all by a straight line, it would be impossible, when the sheets were scattered, to bring the pairs together again, because many pairs would fit one another, but if we made a random zigzag cut through each pair, then it would be possible after separation to bring the pairs together, and the certainty we should feel that we had obtained a pair cut at the same time, would be so much the greater, the greater the number of zigzags and the greater their distinctive peculiarity (fig. 1).

To illustrate secondly the meaning of *negative* correlation, we may similarly imagine a number of *single* sheets of paper cut across by a zigzag cut. When a fit is obtained between two pieces (belonging to an original whole) it is now a *negative* one, i.e., the peaks and depressions of one piece fit the depressions and peaks of the other. And here again the greater the number of peaks and depressions that fitted the greater the certainty that they belonged to an original whole (fig. 1).

Now the method used in calculating the correlation coefficient gives due weight, both to the *number* of zigzags, and to the *magnitude* of the peaks or depressions. The symbol used for the correlation coefficient is  $r$ , and its numerical value varies from  $-1$  to  $+1$ .  $r = 0$  means no fit,  $r = +1$  means perfect positive fit,  $r = -1$  means perfect negative fit.

Except in artificially chosen examples, we cannot expect perfect

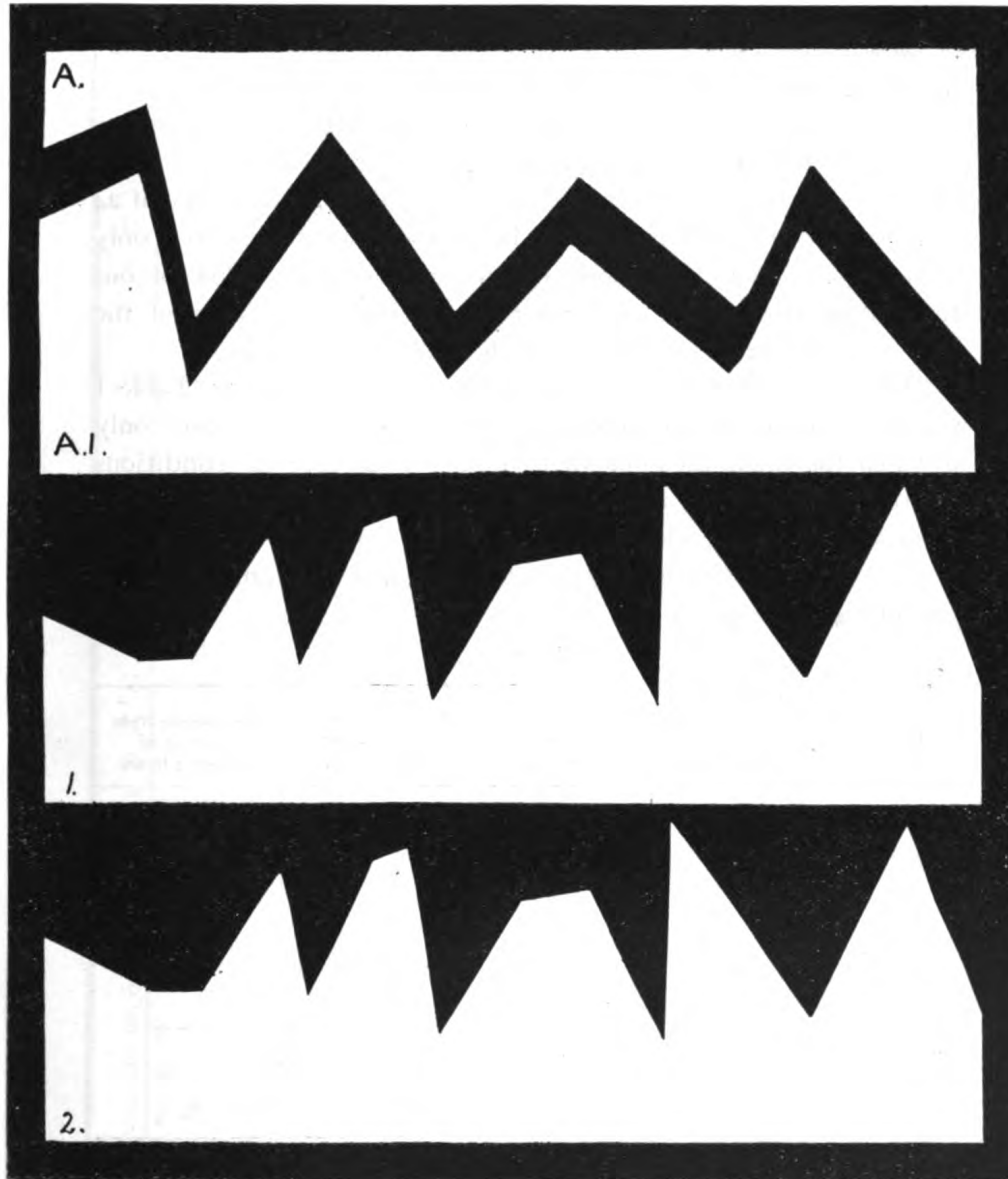


FIG. 1. To illustrate correlation or fit.—There is a *negative* fit between the two pieces of paper A and A.I.—i.e., when A is inverted, as in the figure, the depressions of A fit the peaks of A.I. and the peaks of A fit the depressions of A.I. There is a *positive* fit between the two pieces of paper 1 and 2—i.e., the peaks of 1 fit the peaks of 2, and the depressions of 1 fit the depressions of 2.

fit, because we always have errors of observation, and errors due to random sampling, but the nearer we get to 1, positive or negative, the greater our belief in a connection.

Now the fit in the figures for quinine and rigor is very close,  $.92 \pm .04$  (instead of 1). As we said before, in obtaining a positive fit between two pieces of notepaper we should feel the more certain that they were cut at the same time the greater were the number of zigzags (and the greater their distinctive peculiarity). So that if 24 zigzags fitted, the certainty would be greater than if there were only 12 zigzags. Our cases, however, are insufficient to allow of our distributing the quinine and rigor figures over every hour of the day, i.e., into 24 observations, which would be desirable.

We have said above that the fields of observation in Table I were not coincident, we now proceed to consider those cases only in which the fields are coincident, i.e., in each case the conditions affecting the taking of the quinine and the onset of blackwater are, so far as we can tell, identical.

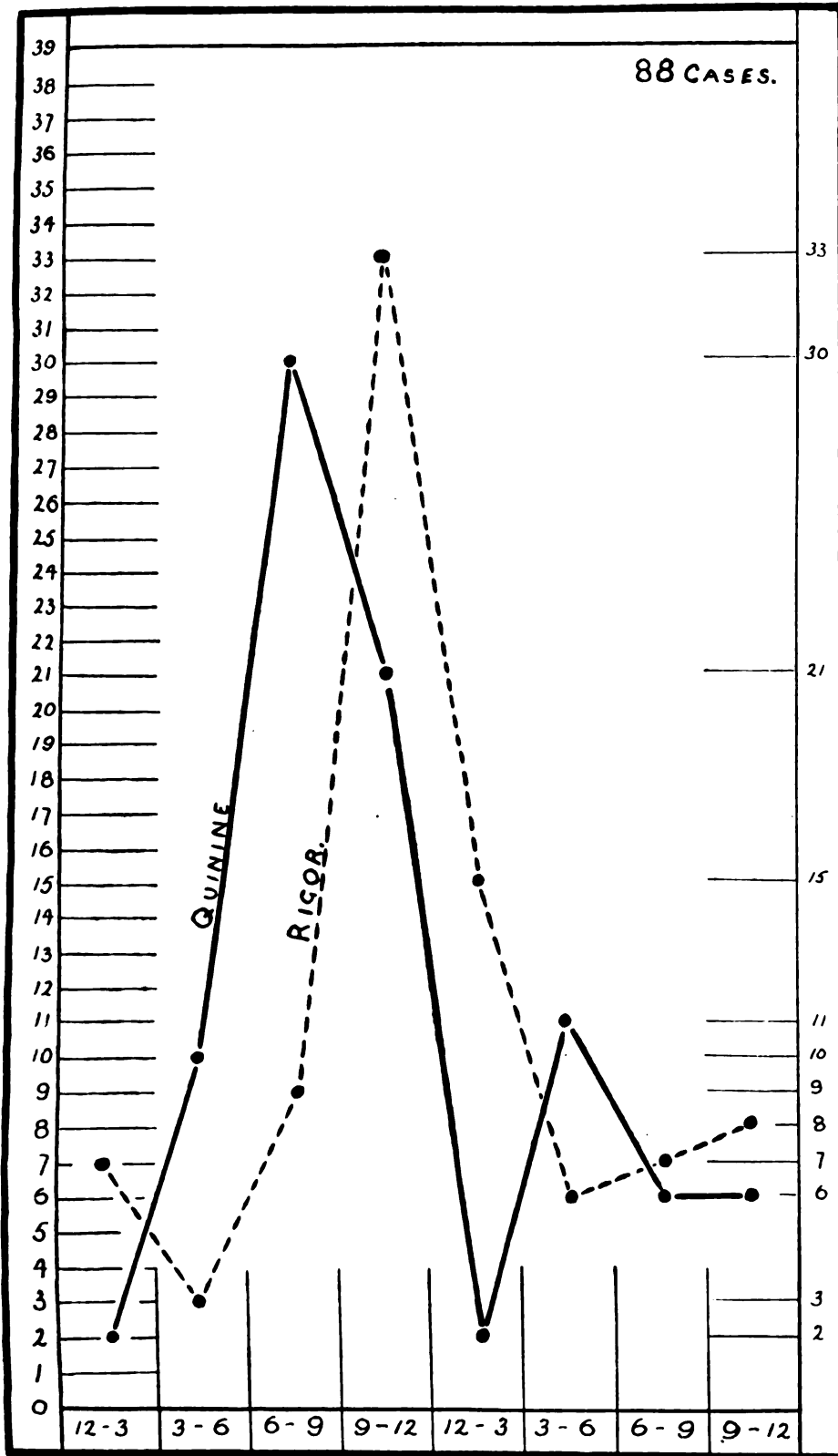
(1) We have 88 cases in which both the time of quinine and the time of rigor are given.

TABLE II

Hour	Number of people taking quinine at stated hour	Deviations from average of 11 per 3 hours	Number of rigors occurring at stated hour	Deviations from average of 11 per 3 hours	
A.M. {	12— 3	2	— 9	7	— 4
	3— 6	10	— 1	3	— 8
	6— 9	30	+19	9	— 2
	9—12	21	+10	33	+22
P.M. {	12— 3	2	— 9	15	+ 4
	3— 6	11	0	6	— 5
	6— 9	6	— 5	7	— 4
	9—12	6	— 5	8	— 3
	88		88		

The correlation between these deviations *as they stand* is  $r = .34$ , but when the figures for the rigor are moved three hours back the correlation is  $r = .85$ .





F. LEESON, del

CHART I. Showing the relationship of quinine and rigor in 88 cases. The mode of quinine is at 6-9 a.m., that of the rigors is at 9 a.m.-12 noon.

(2) We have 103 cases in which both the time of quinine and the time of blackwater are given.

TABLE III

Hour	Number of people taking quinine at stated hour.	Deviations from average of 13 per 3 hours	Number of cases of blackwater occurring at stated hour	Deviations from average of 13 per 3 hours	
A.M.	12— 3	1	— 12	4	— 9
	3— 6	12	— 1	3	— 10
	6— 9	35	+ 22	9	— 4
	9—12	26	+ 13	30	+ 17
P.M.	12— 3	4	— 7	21	+ 8
	3— 6	13	0	22	+ 9
	6— 9	8	— 5	7	— 6
	9—12	4	— 9	7	— 6
	103		103		

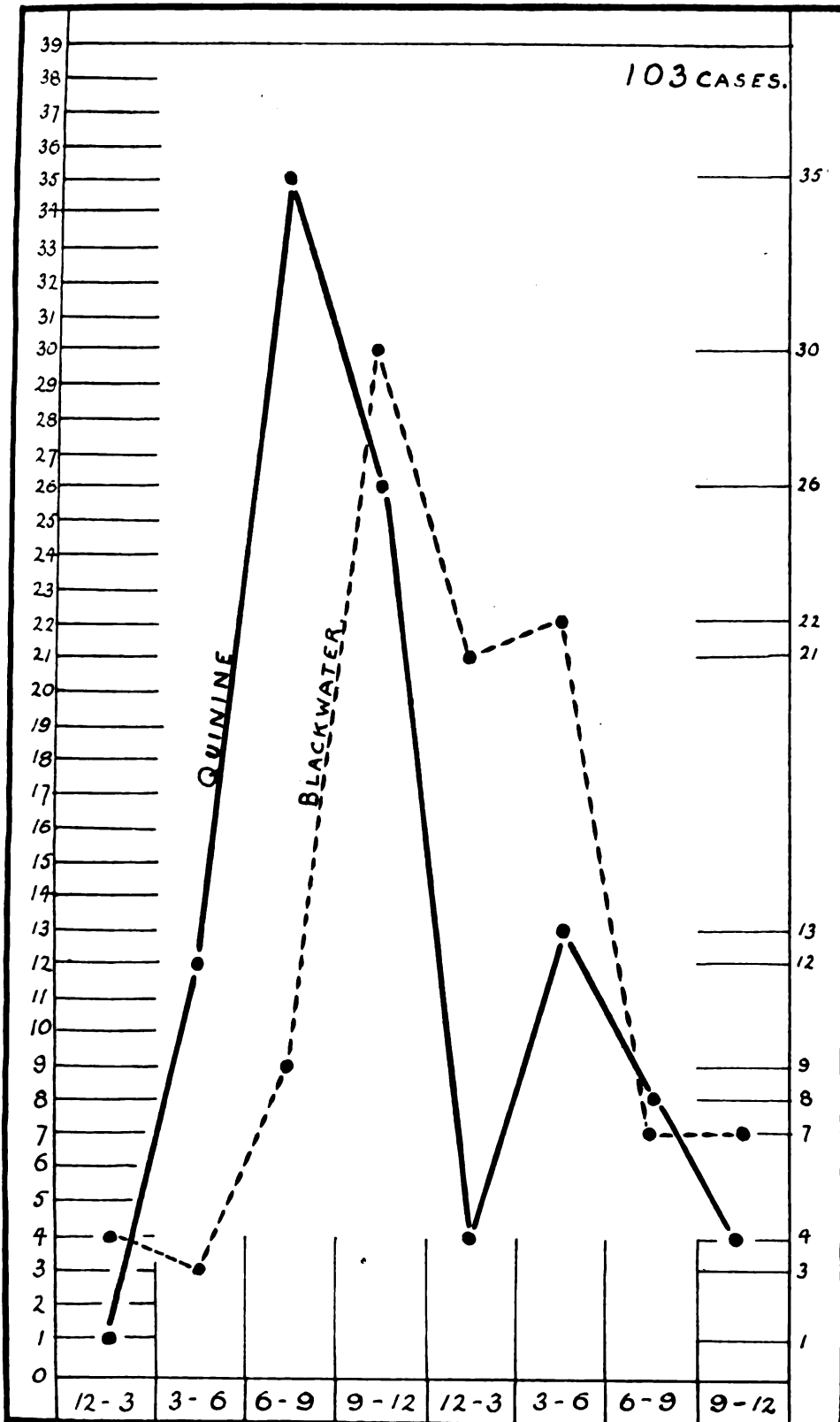
The coefficient of correlation of the deviations as they stand is  $r = .34$ , but when the figures for the blackwater are shifted three hours back the correlation is increased to  $r = .95$  (perfect correlation,  $r = 1$ ).

The increase in the correlations from .34 to .85 in one case and from .34 to .95 in the other, when the figures for the effect (rigor or blackwater) are moved through three hours to fit the (presumed) cause (the quinine) is very significant, and with such a high correlation, .85 in one case and .95 in the other, it is highly probable that there is a connection between the quinine and rigor or the quinine and blackwater, but it is still possible that there is none.

For it must not be assumed that perfect correlation means that we have obtained a *proof* that the events are connected as *cause* and *effect*.

(1) It is possible, for all we know to the contrary, that the mode\*

\* By a mode is meant the most frequent occurrence of any particular fact or event—e.g., if the ages of six persons are 5, 5, 5, 5, 5 and 35 respectively, the average age is 10, but the mode is 5.



F. LEESON, del.

CHART II. Showing the relationship of quinine and blackwater in 103 cases. The mode of quinine is at 6—9 a.m., that of blackwater is at 9 a.m.—12 noon.

(i.e., the most usual time) of the rigors in blackwater, from the nature of the disease,<sup>†</sup> occurs at 9 a.m.-12 noon. If this be so, then of course a strong correlation might exist between it and any other (antecedent) phenomenon that had a mode at some antecedent time. For example, if we investigated the time of taking coffee we should probably find that the mode (i.e., the most usual time) was between 6 a.m. and 9 a.m., and so by moving the figures for the blackwater three hours back we should get a very strong correlation. It is necessary, then, to alter the conditions, either by altering the time at which quinine is taken or by observing a sufficient number of cases when quinine was taken in the afternoon, i.e., in this particular instance where there is no mode for coffee. If, then, we find that any alteration in the time of taking of quinine is followed by a similar alteration in the time of the rigor, the proof of connection is correspondingly strengthened.

(2) It is possible that the modes which we have found do exist for quinine and rigor or blackwater, although they exhibit a high correlation, yet are not really related to one another as cause and effect. Both modes might be due to some other cause or causes; for instance, the rise in the price of wheat and the rise in the male death rate (between which there might be a strong correlation) might be due to the war, and yet we could not argue that the rise in the price of wheat was the cause of the rise in the male death rate.

To sum up, we have really started with the assumption that the taking of quinine by persons with a certain diathesis is the cause, or a cause, of 'blackwater.' On this assumption we should expect to find a positive correlation between the time of taking the quinine and the symptoms of the illness.

We do find such a positive correlation, and as the effect follows the cause usually after a certain period of time, we should expect the correlation to be brought nearer to unity as the figures for the effect are brought more in coincidence with the cause, in technical terms

<sup>†</sup> We find, for instance, the following statements with regard to malaria in Mannaberg (1905):—  
'Maillot and the majority of observers since him state most paroxysms (about two-thirds of all cases occur, between midnight and midday—in other words, in the morning.' Moreover, according to Maillot, 'the greatest number of quotidian parasites occur about 10 a.m.; the smallest number between 9 p.m. and midnight.' Again, it is stated that 'Maurel observed in Guiana the majority of paroxysms between 2 and 5 p.m.' Mannaberg himself states 'that out of 107 cases 91 per cent. of the paroxysms occur during the period between 10 a.m. and 3 p.m.' We have not so far been able to examine these statements critically, so can form no estimate as to what they are worth.

as we bring the two modes together. This also we find. Had the quinine prevented blackwater we should have expected a large negative coefficient, and if the quinine had no effect we should have expected the correlation to be practically nothing.

From what we have said, there are then three possible explanations of this correlation which we have found to exist between quinine and blackwater.

(1) It is purely accidental, due to the fact that blackwater has a 'natural' mode in the 9 a.m.-12 noon interval. This, as we have said, could be eliminated by seeing whether the same mode existed when quinine was taken at other times than 6-9 a.m. For this purpose we require further cases with the times accurately recorded.

(2) The correlation is produced by some unknown cause or causes which determine both the fact that the mode of quinine is at 6-9 a.m., and that the mode of the rigors is at 9 a.m.-12 noon. We cannot suggest any such cause.

(3) That the correlation is one of *cause and effect*.

We believe, then, that, provided (2) can be excluded, that an examination of further cases, in the way we have done here, will decide whether (1) or (3) is the true answer to the question.

By applying the statistical method adopted in this paper, we thought it possible that a solution of the question might be forthcoming, and although the cases we have been able to obtain are neither sufficiently numerous, nor recorded with the necessary accuracy of detail for us to claim that a solution has been arrived at, yet we believe that as a result of our analysis the problem can now be approached, when further cases are forthcoming, in such a way that we may fairly anticipate a solution in the near future.

That this is a matter of vital importance, and not simply one of scientific interest, everyone who has any knowledge of the disease must realize.

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# GANGOSA IN NEW GUINEA AND ITS ETIOLOGY

BY  
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PLATES XIV-XVII

Gangosa, or Rhinopharyngitis mutilans, is a disease of limited geographical distribution, being, according to our present knowledge, confined mainly to the Pacific Islands.

According to Castellani and Chalmers (1910), cases of this disease were described first in 1828 by a Spanish Commission to the Marianne or Ladrone Islands, under the name of Gangosa, meaning 'Nasal Voice.' Fordyce and Arnold (1906), and in the same year Leys, gave a clinical account of a similar disease in Guam, and were followed by Mink and McLean (1906) who discovered cases of Gangosa in the Ladrone and Caroline Islands.

Later, Stitt (1907) reported the occurrence of Gangosa in a white man, who was treated in the United States Naval Hospital at Canacao. This patient had contracted his infection during a stay in Guam, where he had become intimately associated with natives in whose families Gangosa had been known.

A further case with complete post-mortem findings was described by Musgrave and Marshall (1907) in a native of Santo Domingo de Basco, Batan Islands, being the first case reported from the Philippines. Careful examination of the blood was made by these authors. According to their findings the blood did not show any marked pathological changes; the differential count proved that the relative numbers of the various types of leucocytes were normal.

In 1909, N. T. McLean observed two patients suffering from what he believed to be Gangosa on the occasion of a visit

to the Hospital at Gonaives, Haiti, and stated that according to information that he collected numbers of such cases were supposed to have occurred at Port au Prince.

A case was also published by Stitt in a young Filipino who had never been outside Cavite Province (Philippine Islands).

In 1910 Garrison pointed out that there were 327 cases of Gangosa in Guam, and pronounced the opinion that according to his experiences from a clinical, and particularly from a therapeutical standpoint, Gangosa was to be considered a very late form of Syphilis—perhaps a fourth stage. Odell (1911) reported a positive Wassermann reaction in 82% of his cases. Anti-syphilitic treatment seemed to benefit all cases considerably.

Rossiter (1912) described a disease in a two year old child where the destruction of the hard and soft palate were the most prominent lesions. In specimens taken from the ulcerated surfaces numerous *Spirochaeta pertenuis* were found. Two months previously the child had suffered from typical framboesia.

Also in 1912 Ziemann drew attention to the fact that a disease resembling Rhinopharyngitis mutilans occurs not infrequently amongst the negroes of Kamerun.

I observed cases of Gangosa for the first time in the Torres Straits Islands, whilst on a short journey during the latter end of 1910.

Then I was inclined to consider this disease either as a peculiar manifestation of syphilis or as a hitherto undescribed disease occurring on Murray Island. Elkington referred to most of these cases in the yearly report of 1912, adding a number of cases which he discovered on Darnley, Boigu, Saibai and Dauren Islands. The disease must have been prevalent on Murray Island for nearly a century, as it is, according to Elkington, referred to by Dr. Wilson, R.N., in an account of a visit made by him to Murray Island in 1822.

The occurrence of cases of Gangosa in New Guinea has been noted previously, but these cases had been regarded by Sir William Macgregor as lupus.

During my short journey in New Guinea typical cases of Gangosa were seen in villages on the south coast, especially in Kerapuna, a fairly large village, where as many as ten cases were

examined. Furthermore a number of natives suffering from Gangosa were encountered in the western parts of British New Guinea and in the Mekeo District, and there the earliest stages of the disease were seen, showing peculiar manifestations which did not altogether coincide with the description given by previous observers elsewhere.

#### CLINICAL ACCOUNT OF THE DISEASE

According to Castellani and Chalmers the disease 'begins as a sore throat, when on examination a nodule can be seen on the back of the pharynx, the posterior pillars of the fauces or the edge of the soft palate. This ulcer usually spreads rapidly at first, but more slowly later, and destroys the soft palate first, later the bony parts of the palate and the nasal septum. In consequence the skin of the nose falls in, and the cavity of the nose and mouth are connected.

'In consequence of the destruction of the soft and hard palates the ulceration may extend afterwards to the face or lips or affect the pharynx. As a rule a pungent odour is given off, whilst a slight discharge of granular and necrotic débris is present. The ulceration may progress continuously for a period of 10-35 years, or it may advance at certain times and be quiescent at others, or it may cease at any time, leaving a chronic ulcer.'

So far as observed, the cases of Gangosa met with in New Guinea may be conveniently divided into three groups.

*Firstly.* Early cases in which the patients had been ill for a few months only and where a small area of the skin of the face was found to be affected. The cartilaginous septum had been destroyed but the disease had not caused any extensive destruction of the bone.

*Secondly.* Cases in which the ulceration had advanced further. Large areas of the face were found to be the seat of extensive weeping sores. The bone near the affected parts had become implicated, and the disease had led to the destruction of the skeleton of the nose and the hard palate.

*Thirdly.* Cases in which the active ulcerating process had come to a standstill. Extensive cicatrisation had taken place at the

seat of the ulceration, as a rule the nose had disappeared, and was represented by a smaller or larger opening. In one extreme case the opening was so small that the woman in question was able to smoke comfortably a cigarette through the small opening—the only remains of the nose. In another case the whole of the back of the pharynx could be seen without any difficulty through the large opening in the middle of the face.

The uncertain etiology of Gangosa and its similarity to tertiary syphilis in its clinical manifestations makes it advisable to refer in greater detail to the history of a few of the typical cases met with.

### EARLY CASES

Only a comparatively small number of early cases were seen.

CASE 1. (Pl. XIV, fig. 1.) Apau Kaianga was an unmarried girl of about 12 years of age. Her parents as well as four brothers and sisters were alive and well. Her disease began about one month previous to my visit as a sore below her nose which spread rapidly, affecting especially the septum, the left ala of the nose, the upper lip and the left corner of the mouth, and had already destroyed part of the cartilaginous septum.

The examination revealed the nose considerably flattened, and also thickened on account of oedematous swelling. The left ala was the seat of a large raised dry ulcer, covered by a thin scab, below which there was a reddish uneven granulating surface. This ulceration had extended to the upper lip, being confined to its central part, just below the nostrils. The left corner of the mouth was the seat of a similar ulcer which showed an uneven surface. The upper lip around the ulceration was swollen and oedematous and its surface was intersected by purplish irregular raised lines simulating scar tissue. The cartilaginous septum had been partly destroyed by the ulceration, whereas the osseous septum was still intact. The hard and soft palate were of normal appearance.

CASE 2 (Pl. XIV, fig. 2.) Ame Aua was a woman about 25 years of age, belonging to the same village. Her father was still living, her mother had died. Some time ago she was married, her three children were alive and well, except for manifestations of Yaws.

According to her own statement she had been ill for four months only, the disease in her had begun as a small sore on the tip of her nose which had spread gradually into the nasal cavity. The examination revealed a fairly large ulcer on the nose, a yellowish greasy scab covering the raw granulating surface. The cartilaginous septum of the nose had been destroyed by ulceration and the nose was, in consequence, flattened. A similar ulcer below the nostrils extended into the nasal cavity. On the right cheek was a narrow raised line resembling oedematous scar tissue. The hard and the soft palate and the pharynx did not show any pathological changes.

CASE 3. (Pl. XIV, fig. 3.) The woman belonged to Kerapuna, a large village on the south-east coast of New Guinea. She had been married and was the mother of six children. Four of them were healthy and two were in the secondary stage of Yaws.

Her upper lip showed the same scar formation as described in the two previous cases, the scars being oedematous and raised in the centre. The free edge of the skin of both the alae showed ulceration and both nostrils were closed up by yellowish scabs.

CASE 4. (Pl. XIV, fig. 4.) Ame Kwipa, a young girl, belonged to Veipa, a large village in the Mekeo District. She had had small sores on her face, especially on her right cheek for about one year, and these had shown a tendency to heal during the last two months. Her brothers and sisters were healthy.

When examined her nose appeared broadened, its skin swollen and oedematous. The upper lip, especially the right side, was swollen, oedematous, and showed raised irregular lines resembling scars. The right cheek was the seat of a number of small pustules which contained whitish pus.

CASE 5. (Pl. XV, fig. 5.) Ainye, a girl about 14 years of age, belonged to Bebeo, a small village in the Mekeo District. Her father was alive and well, her mother and one brother had died. Her illness commenced about one year previously with a small sore on the upper lip below the nose, which had spread slowly and gradually to the nose and downwards to the mouth.

When examined her nose was flattened, showing an uneven surface; the mucous membrane of her nostrils was covered by a yellowish scab. The upper lip was swollen and succulent in appearance, whilst the left angle of her mouth was drawn up by the formation of scar tissue. Her body was clean.

CASE 6. (Pl. XV, fig. 6.) The patient was a young unmarried girl, Wabnagi, belonging to Kaile, a village on the south-east coast of New Guinea. Her mother was alive, her father dead. Her disease began about two years previously as a small sore on the upper lip just below the nose, which had spread gradually to the cheeks and into the interior of her nose.

At the time of examination her nose was flattened and had sunk in, as the cartilaginous septum had completely disappeared. The surface of the nose was uneven and rough; the right half of the upper lip was drawn up on account of extensive scar formation. The same scar formation was seen on both cheeks, more extensively, however, on the right side. The skin of the affected parts was very thin and shiny, intersected by raised streaks of apparently dense scar tissue. Here and there on the nose and cheeks were small ulcerations covered by a yellowish scab. The hard and soft palate did not show any pathological changes.

CASE 7. (Pl. XV, fig. 7.) Was a young unmarried woman belonging to Kerapuna. Her parents as well as two younger sisters were alive and well. Her disease began when a small child with sores on her face which had since spread, implicating the nose. She showed large and extensive ulceration on her face; the nose had completely disappeared and was represented by a small opening in the middle of the face surrounded by irregular ulcers covered by yellowish scabs. The upper lip was partly destroyed by extensive ulceration. A large ulcer on her left cheek had a raised granulating surface and irregular edges, secreting copiously a slightly yellowish clear, foul-smelling, discharge. The left cheek showed scar formation. The hard and soft palate were normal.

### LATER CASES

CASE 8. The patient, a man about 22 years of age, belonged to Hula, a village on the south-east coast of New Guinea. His mother had died, his father was alive and healthy. He was married and had three normal children. According to information the disease had commenced about five years previously with a sore on the forehead over his left eye-brow, which had spread gradually until the greater part of his face was affected. His face, when examined, was badly disfigured; the nose was flattened and was sunk in. Deep scar formation on the right side of his forehead caused the upper eyelid to be drawn up. The skin on the left side of his forehead showed extensive ulceration which had spread downwards and caused the destruction of his left eye. The central part of his upper lip was partly destroyed. The hard palate was perforated, showing a round hole of about 2 cm. in diameter, with smooth rounded edges.

CASE 9. Was a woman, Kila Alukma, of about 40 years of age, living in the same village as the previous case. Her parents were dead, her two daughters were married and apparently well.

Her disease had begun about ten years previously 'with a pain in the stomach which gradually worked up to her mouth and came out through her nose.' She pointed out that there had been a sore in her mouth before her face became affected. This case was very similar in appearance to Case 8. There was extensive scar formation on the face round the nose, the upper lip was drawn up on account of dense scar tissue. A stinking discharge from the nostrils was noticed; the hard palate was perforated, the oral and nasal cavity communicating through a round opening of about 3 cm. in diameter.

CASE 10. Was an unmarried woman of about 35 years of age belonging to Kaile on the south-east coast of New Guinea. Her mother was still alive, her father was dead. About ten years previously a small ulcer had started on her nose and soon began to spread. At the same time ulcers appeared on her legs.

When examined the cartilaginous septum of her nose had disappeared and extensive scar tissue formation was observed around the nose, on both cheeks and on the upper lip, the mouth and eyelids being normal. The hard palate was perforated. Her legs, as well as her left forearm were the seat of deep ulcers of varying size some of which extended as far as the bone, whilst some of them were covered by a greyish greasy scab.

CASE 11. (Pl. XV, fig. 8.) Was a man, Aiaba, about 40 years of age belonging to Kivori village. Both his parents were dead. He was married and the father of six healthy children. His illness had begun about four years previously with a small sore on the upper lip just below the nostrils, which spread at first into the nasal cavity and later to his face. He had lost the toes of his right foot in an accident when he was a small boy.

The patient's face was of horrible appearance. The nose had completely disappeared, leaving a large hole in his face through which the posterior wall of the pharynx could be seen. A large irregular granulating sore on his forehead was covered by a yellowish dry scab under which thick pus had collected. A similar sore was on his right cheek showing here and there recent scar formation. The upper lip was the seat of extensive scar formation. The skin of his left cheek was swollen and oedematous.

The hard palate was perforated and the oral cavity communicated with the nasal cavity by means of a large round opening with sharp edges.

### THIRD STAGE OF THE DISEASE

CASE 12. (Pl. XVI, fig. 9.) A woman, Ugre, about 25 years of age, who lived in Kerapuna, a village on the south-east coast of New Guinea. She had been ill, according to information, for about ten years; her nose had been affected long before her mouth became involved. When she fell ill her husband married a second wife. Both were seen and were in excellent health. When examined the skeleton of her nose had disappeared and there was a hole in the centre of her face, the surrounding skin as well as the skin of the upper lip and forehead showed extensive scar formation, indicating that the whole face had been previously affected by the disease.

CASE 13. (Pl. XVI, fig. 10.) An unmarried woman, about 32 years of age, was seen in the same village as the previous case. Her father had died, her mother was still alive and in good health. Her disease had started when she was quite a small child. When examined the bony skeleton of her nose had completely disappeared, the nose was sunk in so that the nostrils were only represented by a small opening. Both the upper and the lower lips had disappeared, the skin of the face directly joining on to the gum, so that the teeth were unprotected; the skin of her face was one mass of scar tissue, the right lower eyelid was drawn down by scars giving rise to an ectropion. The left side of her throat and her left shoulder were of the same appearance as the skin of her face, showing extensive scar formation. Her soft and hard palate did not show any pathological change.

CASE 14. (Pl. XVI, fig. 11.) An unmarried woman of about 35 years of age. She had been ill for quite a long time, in all probability between ten to fifteen years. Both her parents were alive and in good health. Her disease began on the 'outside of her nose' in the form of a small sore which had spread slowly implicating the lips and mouth. Some time ago the ulcerated parts had begun to heal up. Her face was completely disfigured. The whole outer nose had disappeared as well as the anterior part of her hard palate, so that the oral and nasal cavities communicated extensively. The posterior part of the hard and soft palate were still intact. The central part of the upper lip and the lower lip had disappeared, the outer skin joining immediately on to the mucous membrane of the gum. There was extensive scar formation on the skin of her face and on the neck which closely resembled the scars after deep burns.

CASE 15. A woman of about 35 years of age belonged to Ififu (Mekeo District). Her parents were dead, two brothers were alive and well, one of them showing scar formation around the mouth, most probably the remainder of Yaws. She was married and mother of a healthy girl. Her illness began when she was a girl, in the form of a small sore on the upper lip. Subsequent to the infection of the face a large ulcerating sore developed on the abdomen, around the navel which, however, soon healed up. Her mouth was small and deformed, the left angle drawn up by scar tissue. Extensive scar tissue formation was seen on the upper lip and both cheeks, the skin otherwise being smooth, shiny and very thin.

CASE 16. (Pl. XVI, fig. 12.) Was an unmarried woman, Kaiyo, about 35 years of age. Her parents were dead. The disease began at a very early age with a sore on the left foot soon after she had begun to walk. Later on a small ulcer appeared on her upper lip below the nostrils which gradually spread, eating away the whole upper lip and the nose. At the time of examination, her nose as well as her upper lip had completely disappeared, a small round hole representing

the nasal opening. Extensive scar formation was noticeable around the mouth and on the cheeks. Her left eye had been destroyed by the disease. The hard and soft palate did not show any pathological changes.

CASE 17. Was a middle-aged woman. Her parents were dead. She had four brothers and sisters, two of whom had died. She had never been married. Her disease began some time ago with an ulceration inside her nostrils. Her nose had been destroyed by the disease, and the back of her pharynx was visible through a large triangular opening in the face. The anterior part of the hard palate was destroyed, but the posterior part was still existent. Her mouth was deformed, the right angle drawn up by scar tissue.

The case histories recorded above describing a few typical cases out of the many examined, prove that Gangosa, or Rhinopharyngitis mutilans, is a definite morbid entity, and can be differentiated from other diseases causing similar lesions, such as Syphilis, Lupus and Leprosy. It is a very chronic but very rarely fatal complaint. A great number of the patients recover without specific treatment, mostly, however, after the morbid process has brought about extensive destruction of the face.

From the number of cases observed in the different stages, Gangosa in New Guinea seems to differ in its earliest stages from the disease described by Castellani and Chalmers.

According to my experience in New Guinea, Gangosa usually begins as a small ulcer on the upper lip, just below the nose. Only in a small number of cases did patients state definitely that the palate was affected before any other part of the face. This small ulcer soon begins to spread, destroying at first the fleshy parts of the face, such as the lips, the alae and the skin of the cheeks surrounding the nose. As a rule, the tissue in the near neighbourhood of the ulcer is swollen on account of the oedematous infiltration, the surface skin is glossy and intersected by reddish, raised lines, resembling in some respects a relief map of mountains.

In the earliest stages the ulcer may spread gradually or sometimes rapidly, finally implicating the bones of the infected regions, destroying at first the cartilaginous parts of the nose, and later on the bony skeleton and the soft and hard palate.

The ulcers are raised, possess irregular edges, and are not well defined from the surrounding tissues. The surface of the ulcer is formed of granulating tissue, which secretes a malodorous yellowish discharge, and is often covered by a dark yellowish scab underneath which pus accumulates. Very soon, however, some of the



ulcers show a tendency towards healing. The granulation tissue begins to discharge less, and after a varying period new smooth skin grows over the ulcer from the surroundings, often, however, breaking down again, and giving rise to renewed ulceration.

Some time afterwards dense scar tissue is formed, leading to the deformities of the face so characteristic of the disease.

Now and again only a single primary ulcer is seen, at other times a number of ulcers appear simultaneously.

The morbid process may come to a standstill at any stage of the disease, even before it has led to extensive destruction.

The tendency to scar formation is a marked feature of the disease, and in this respect Gangosa simulates Syphilis, so that in some of the late cases a differential diagnosis would be practically impossible.

Some of the patients who showed typical symptoms of Gangosa in their faces were suffering from large ulcerations on other parts of the body, especially on the legs. It is impossible to decide whether these ulcers corresponded only to *Ulcus tropicum*, or were due to the same parasites as the face lesions.

It is interesting to note that the great majority of cases seen were women, only a comparatively small number of men being affected.

#### THE ETIOLOGY OF THE DISEASE

As the etiology of the disease is unknown, material was collected for microscopic examination. Special attention was paid to the early lesions with a view of finding a possible parasite in the oedema fluid. It may well be understood that the examination of the secretion of open sores of advanced cases did not seem to offer much opportunity for the forming of a definite opinion; smears taken from the secretion of open wounds always contain enormous numbers of bacteria and spirochaetes of varying shapes and sizes, which are to be considered as secondary infections.

From the earliest cases showing the marked oedematous swelling of the affected parts as described above, oedema fluid was obtained by pricking the skin over the lesion with a needle in the same way as serum would be obtained from a leprous nodule. The specimens

were stained with Giemsa's stain. In the oedema fluid of five early and two later cases, in addition to a small number of red and white corpuscles, small bodies were found resembling yeast cells. The majority of them occurred free, only a small number was contained in leucocytes (Pl. XVII, figs. 1 and 2). These cells showed a marked polymorphism; some of them were round, others oval or pear-shaped (comp. figs. 3 and 4), and were seen either singly or in groups.

The cells measured from  $0.75\ \mu$  to  $4\ \mu$ , and consisted of a lightly bluish staining cytoplasm showing a typical network structure, a great number of them contained a distinct and sharply-defined vacuole (Pl. XVII, figs. 5-7) and small roundish masses which resembled chromatin in their staining reaction. These chromatin-staining masses occurred either singly, being more or less sharply defined, roundish or oblong in shape (figs. 7, 8, 13) or as number of similar bodies enclosed within the cell (figs. 9, 10, 14, 15).

Budding of the cells in its various stages could be frequently observed.

In the earliest stages only a small protuberance on the periphery of the cell was seen, consisting of cytoplasm only (figs. 3a, 6, 14).

In the more advanced forms this protuberance had increased in size, and at this stage a small chromatin-like staining mass was often seen at the base of the bud (figs. 3b, 16). Sometimes the bud itself contained one or more chromatin-like staining particles (figs. 7, 13).

The bud increased in size, and usually became detached before it reached the same size as the parent cell (figs. 9, 10, 16), and in this case invariably contained chromatin-like staining particles and often a distinct unstained vacuolic area (fig. 18).

In many instances only one bud was formed, not rarely, however, two, three or even more buds were seen still connected with the cell (figs. 7, 15).

Forms as seen in fig. 17 were noticed only very rarely. In these forms the cell consisted of a central portion stained lightly reddish by Giemsa's method, containing one or more distinct chromatin-like particles, the whole being surrounded by a thick bluish pellicle.

From their morphological appearance there is no doubt that the

cells described are to be considered as parasites belonging to the genus *Cryptococcus* of the family *Saccharomycetes*. The characteristic features of *Cryptococcus* is the reproduction by budding only and the absence of endospores and ascospores.

In these parasites only multiplication by budding was observed, endospores have never been seen, although carefully sought.

The parasite itself has all the typical features of yeast cells. Unfortunately circumstances only permitted of the collecting of dry films, which were stained by Giemsa's method, and nothing definite can be said about the nuclear details. When stained by Gram's method the cells did not become decolourised.

Parasites of the genus *Cryptococcus* are well known to produce granulating sores in man and beast; the best known and most studied disease due to *Cryptococcus* is probably epizootic lymphangitis in horses, occurring in different parts of the world.

Gilchrist and Stokes (1898) describe a similar parasite in man from a case of chronic ulcerative dermatitis, resembling Lupus, under the specific name of *Cryptococcus dermatitis*. Busse (1894) isolated *Cryptococcus hominis* from a purulent periostitis of the tibia of a woman.

On the other hand, yeast cells occur very frequently in open sores of every description.

These parasites were found in great numbers in the oedema fluid of the early cases of Gangosa, where open sores had not formed. Moreover, specimens of the oedema fluid did not contain any other microorganisms besides the yeast cells and a small number of red and white blood corpuscles.

In the seven cases from which oedema fluid could be obtained these parasites were found, most numerous in Case II, where early and late manifestations were present at the same time, and in which the disease seemed to be progressing most rapidly.

The finding of a *Cryptococcus* in seven cases out of eight examined, suffering from Rhinopharyngitis mutilans, justifies the conclusion that one may regard this parasite as the cause of the disease in question.

The fact that blastomycetes are known to cause similar skin lesions in man and animals is an additional argument in favour of the *Cryptococcus* being the etiological agent of Gangosa. We

therefore propose for this parasite the specific name of *Cryptococcus mutilans*.

Unfortunately, circumstances did not allow attempts at cultivation. Animal experiments have been performed at our instigation by Dr. Elkington whilst visiting Murray Island. Two monkeys were inoculated on the eyelid and intra-nasally with the secretion of open sores, but in spite of prolonged observation none of the animals in question showed any lesions whatsoever.

### CONCLUSION

Rhinopharyngitis mutilans is a disease which occurs not infrequently amongst the native population of the south coast of British New Guinea, and is a blastomycosis due to *Cryptococcus mutilans*, n.sp.

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EXPLANATION OF PLATES

PLATE XIV

Figs. 1-4. Cases of Gangosa (*Rhinopharyngitis mutilans*).

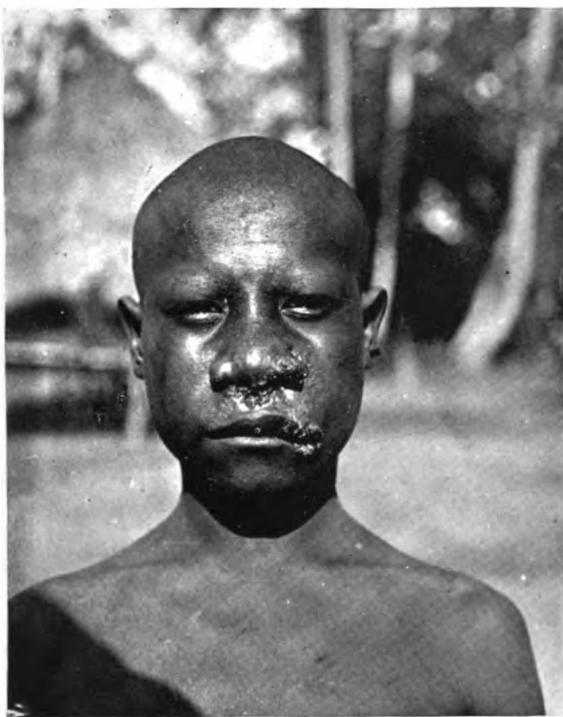


FIG. 1



FIG. 2

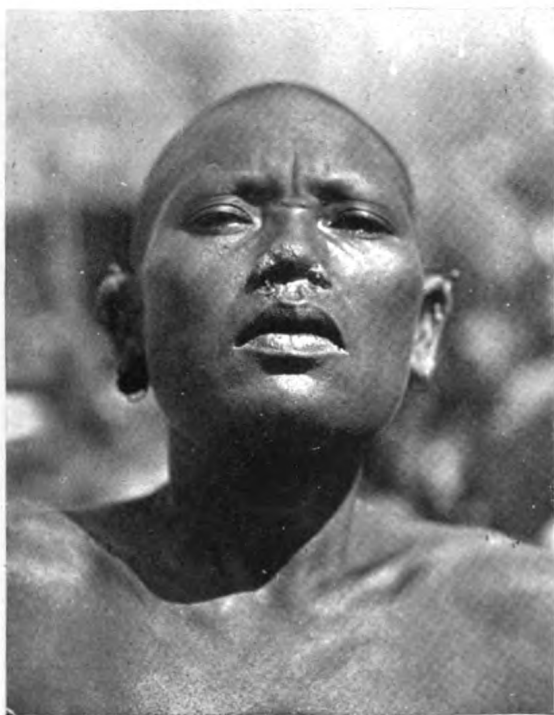


FIG. 3



FIG. 4







PLATE XV

Figs. 5-8. Cases of Gangosa (*Rhinopharyngitis mutilans*)



FIG. 5

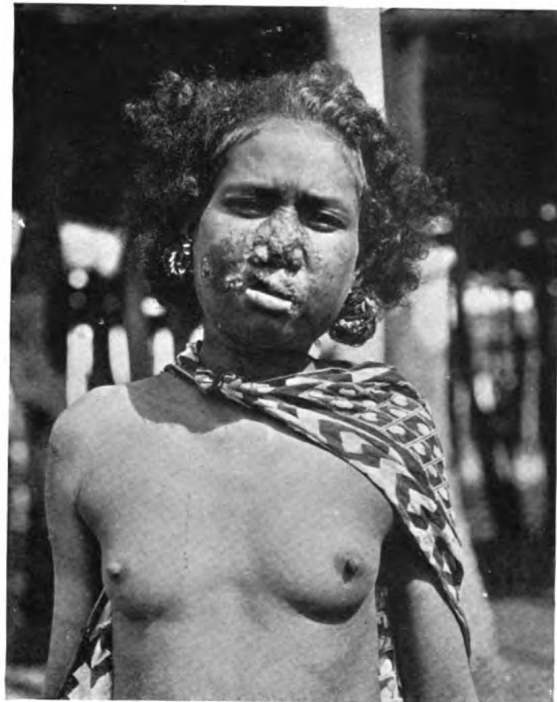


FIG. 6



FIG. 7

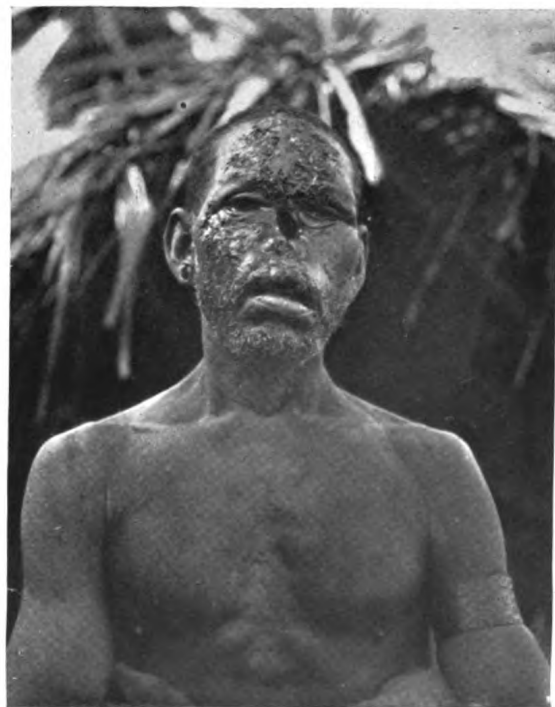


FIG. 8





PLATE XVI

Figs. 9-12. Cases of Gangosa (*Rhinopharyngitis mutilans*).

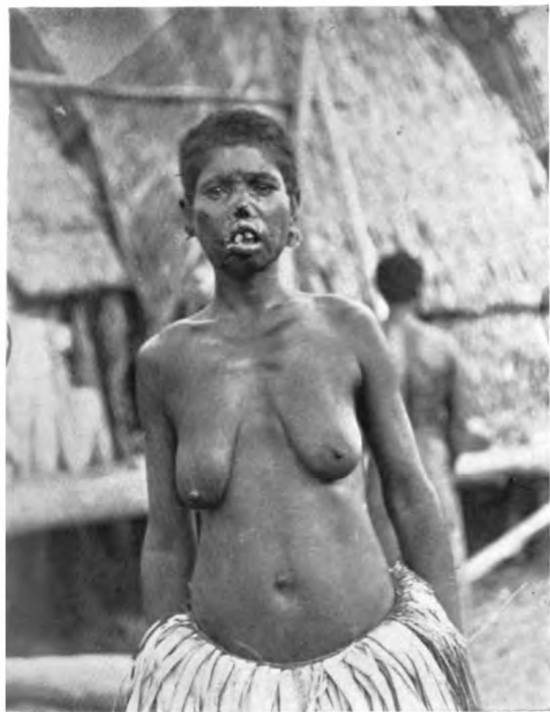


FIG. 9.



FIG. 10



FIG. 11



FIG. 12







## PLATE XVII

All the figures were drawn with a large Abbé drawing apparatus, using 2 mm. apochromatic oil immersion.

For Figs. 1-2. Compensation ocular 8 was used.

For Figs. 3-18. Compensation ocular 18.

The specimens were fixed in alcohol and stained by Giemsa's method.

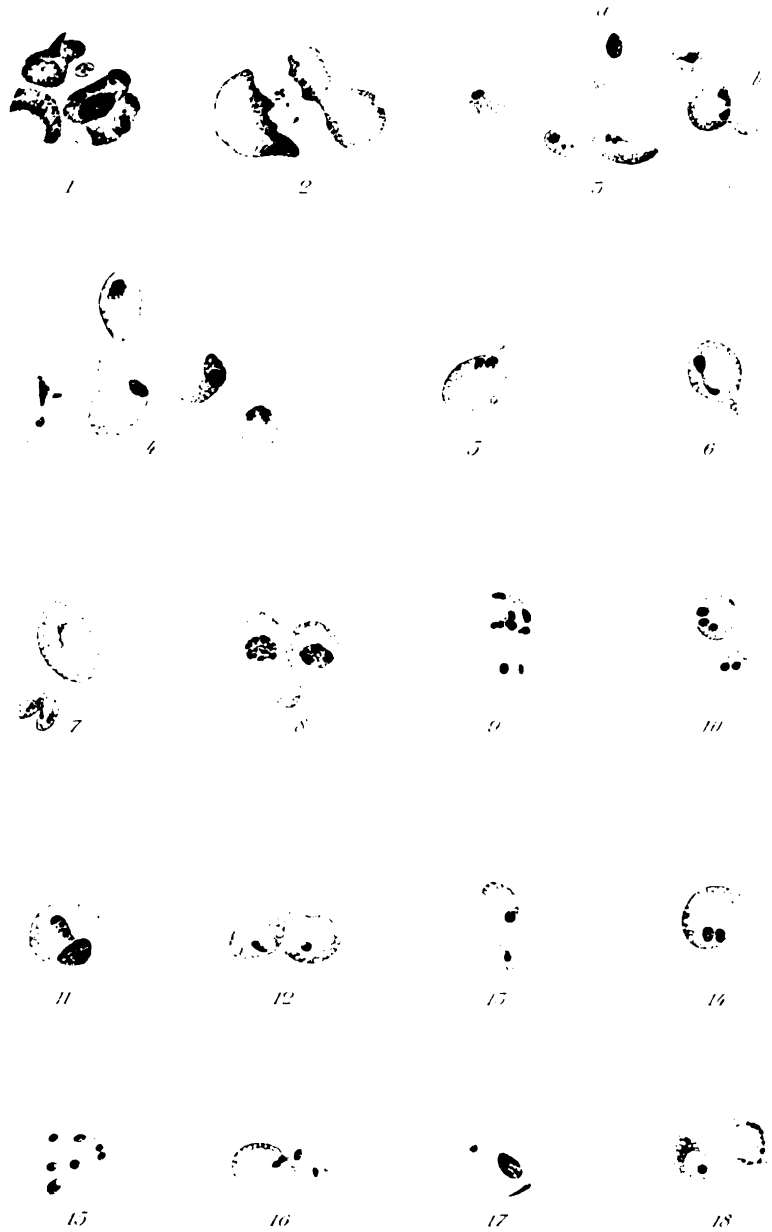
Figs. 1-2. Intracellular forms of *Cryptococcus mutilans*.

Figs. 3-4. Groups of parasites.

Figs. 5-7, 9, 10, 12-16, 18. Different stages of budding.

Figs. 8, 11. Large forms.

Fig. 17. Apparently encysted form, rarely seen.



*Gilulys Roberts, del.*

CRYPTOCOCCUS MUTILANS.



*THEILERIA TACHYGLOSSI* (N.SP.)  
A BLOOD PARASITE OF *TACHY-*  
*GLOSSUS ACULEATUS*

BY

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FROM THE AUSTRALIAN INSTITUTE OF TROPICAL MEDICINE

(Received for publication 20 May, 1914)

PLATE XVIII

Hitherto blood parasites of Monotremes have not been described.

Gilruth, Sweet and Dodd (1911) refer to the occurrence of *Anaplasma marginale* (Theiler) in the blood of Echidnas, but according to their conception the presence of these bodies has no pathological significance.

The blood of an Echidna, from the neighbourhood of Townsville,\* on examination, showed the presence, within the red blood corpuscles, of numerous parasites resembling the *Theileria* of East Coast Fever. On rare occasions the same structures, referred to by Gilruth, Sweet and Dodd as *Anaplasma*, could be seen.

When the blood of the animal was again examined after an interval of a week, most of the parasites had disappeared from the peripheral circulation, and so the animal was killed and films made from the blood and different organs.

Dry films were fixed in alcohol and stained with Giemsa's solution. It was found practically impossible to obtain satisfactory results with wet fixing and staining methods for the blood films. On the other hand the detailed structure of Koch's bodies could be studied to advantage in organ films after wet fixation in sublimate alcohol and staining by Giemsa's method.

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\* I take this opportunity of thanking Mr. J. Humphrey of the Haughton River through whose kindness the animal was obtained.

### MORPHOLOGY OF THE PARASITES IN THE PERIPHERAL BLOOD

In their morphological appearance the parasites resembled closely *Thcileria parva* of East Coast Fever.

They occurred, invariably, within the red blood corpuscles without causing any apparent alteration in the shape and form of the cells.

Bacilliform, ovoid, pyriform, round and comma-shaped parasites were seen, but on careful examination intermediate forms were often encountered. Typical examples of each class could, however, be easily distinguished.

The *bacilliform* parasites (Pl. XVIII, figs. 1-3) occurred most frequently. They measured  $1.8\ \mu$  to  $3.0\ \mu$  by  $0.3\ \mu$  to  $0.8\ \mu$ , and were either perfectly straight or slightly curved.

The cytoplasm stained dark blue and the chromatin was situated either in the centre or at one end of the parasite.

The *comma-shaped* parasites (fig. 5) measured  $2.0\ \mu$  to  $3.0\ \mu$  by  $0.8\ \mu$  to  $1.2\ \mu$ , were broader and were rounded off at one end. The cytoplasm stained less deeply and an unstained vacuolar area was frequently present.

The *ovoid and pyriform* parasites (figs. 4 and 6) measured  $1.0\ \mu$  to  $3.0\ \mu$  by  $0.5\ \mu$  to  $1.2\ \mu$ , and no sharp line of distinction could be drawn between these two types. A vacuole was almost always present, with the chromatin, as a rule, in close proximity to this unstained area. Sometimes, however, the chromatin occurred in the form of an irregular band across the centre of the parasite.

*Rounded forms* (fig. 7) were the least frequently encountered. They measured  $1.0\ \mu$  to  $2.2\ \mu$  in diameter, and resembled the ring forms of malarial parasites. The cytoplasm, which stained light blue, appeared to be condensed peripherally, surrounding a central clear area. The chromatin occurred as an oval or round mass, or was crescentic in shape and situated at the periphery. Sometimes two masses of chromatin were seen in the same parasite.

Multiple infection of red blood corpuscles was very rare. In five slides three corpuscles were found containing two parasites, one contained three parasites and one contained four parasites (fig. 9).

The so-called cross forms with chromatin particles at each pole were seen on two occasions only (fig. 8).

As in infections with *Theileria parva*, the so-called Koch's bodies occurred in small numbers in the peripheral blood, but in great numbers in smears made from the organs, particularly the liver, spleen and lungs. They were either free or included in leucocytes or endothelial cells, and represent in all probability an asexual multiplication of the parasite.

The smallest forms within the leucocytes somewhat resembled cell granules. They were about  $1\ \mu$  in diameter, rounded, with pale blue cytoplasm and a comparatively large chromatin mass in the centre. The largest forms occurring intracellular or free in about the same proportion were rounded or oval in shape, measuring up to  $12\ \mu$  in diameter.

The cytoplasm stained by Giemsa's solution was either dark or light blue, showing the typical network structure; the chromatin occurred in the form of numerous granules, irregular in shape and size and unevenly distributed throughout the parasite. In many instances a great number of chromatin granules were aggregated into masses forming irregular bands across the cytoplasm, at the same time chromatin particles were dotted over the remainder of the parasite (fig. 16).

All intermediate stages in the leucocytes were observed between the smallest forms containing only one small chromatin granule and little cytoplasm and the largest forms with a great number of chromatin granules. The cytoplasm of the largest intracellular forms was usually well defined from the cytoplasm of the host cell; in some cases, however, no such differentiation could be made out, and in these instances irregular chromatin granules were unevenly distributed throughout the host cell. It would seem that, in these cases, the parasite had ruptured, leaving the chromatin granules, most probably surrounded by a very small amount of cytoplasm, free within the host cell. The fact that, now and again, fully developed parasites, of the same type as those found in the red corpuscles, were seen in leucocytes in addition to chromatin granules, suggests that the majority of the chromatin granules may develop into the small blood forms.

As in the case of *Theileria parva*, when the leucocyte contains a large Koch's body the cell nucleus may be disintegrated.

The parasite of the Echidna closely resembles, in its life history, *Theileria parva* as described by Gonder.

It may be assumed from these observations that the chromatin of the small forms which have entered leucocytes divides with a corresponding increase in the amount of cytoplasm.

Finally the larger forms burst, setting free small parasites resembling closely the forms found in the red corpuscles. It seems obvious, then, that the parasite undergoes a definite schizogony within the leucocytes.

A gamogonous and an agamogonous generation, as described by Gonder for *Theileria parva*, could not be followed out in the parasite of the Echidna.

The occurrence of bacilliform parasites, cross forms and Koch's bodies places the parasite of the Echidna in the genus *Theileria*, and the specific name of *Theileria tachyglossi* is proposed.

The Echidna from which the parasites were obtained was infested with ticks—*Aponomma decorosum* (L. Koch)—and was apparently healthy.

#### SUMMARY

A new species of the genus *Theileria* was found in an Echidna, *Tachyglossus aculeatus*.

This parasite, for which the name *Theileria tachyglossi* is proposed, resembles very closely *Theileria parva*. Koch's bodies were found in great numbers in the internal organs, and to a certain extent in the peripheral blood, and represent a stage in the life history of *Theileria tachyglossi* in the warm-blooded animal.

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## EXPLANATION OF PLATE XVIII

All the figures were drawn by means of an Abbé drawing apparatus from preparations fixed dry and stained with Giemsa's solution. Zeiss apochr. homog. 2 mm., comp. ocular 18 except figs. 15 and 16, in which comp. ocular 8 was used.

Figs. 1-3. Bacilliform parasites.

Fig. 4. Ovoid parasites.

Fig. 5. Comma-shaped parasite.

Fig. 6. Pyriform parasite.

Fig. 7. Round parasite.

Fig. 8. Cross-form parasite.

Fig. 9. Four parasites in one corpuscle.

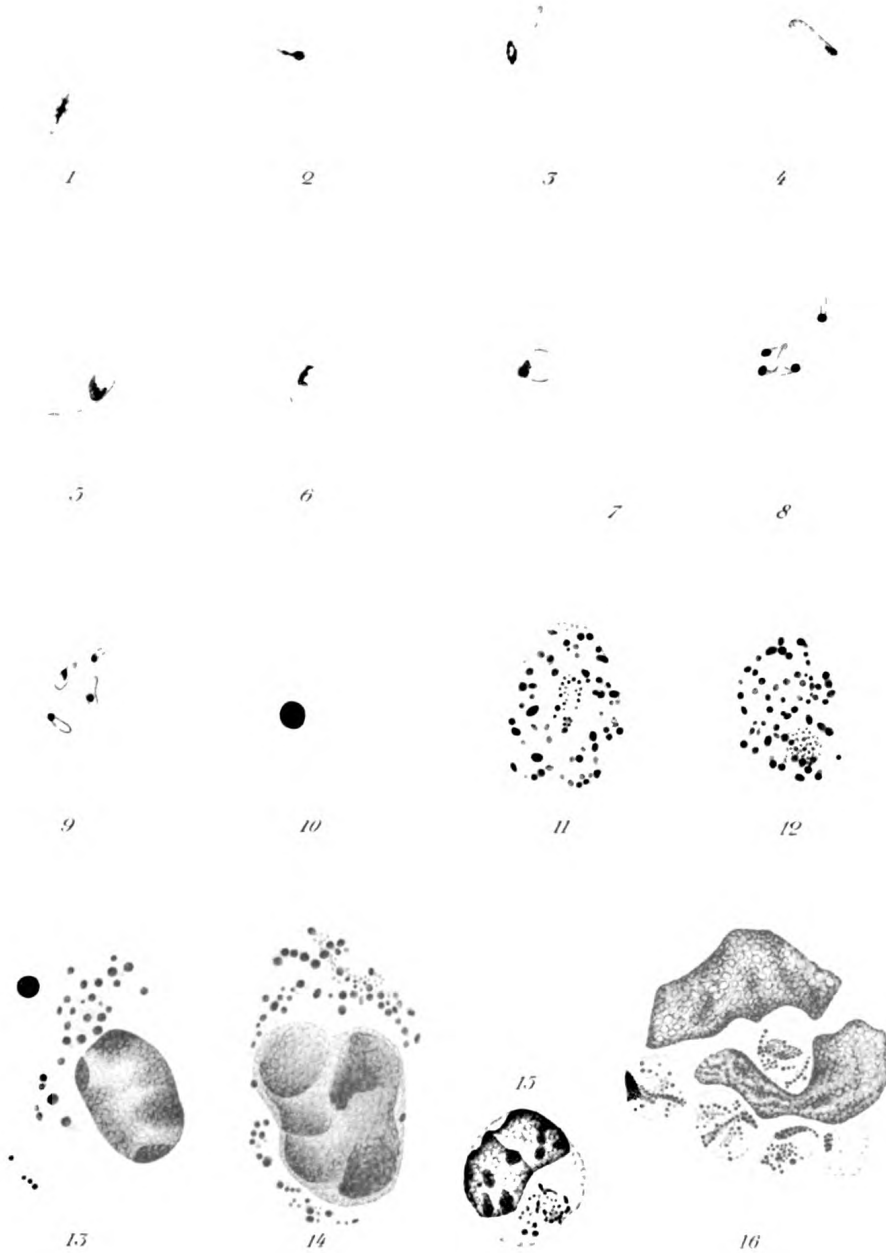
Fig. 10. *Anaplasma*.

Fig. 11. Koch's body, large free form with deep-staining cytoplasm.

Fig. 12. Koch's body with pale-staining cytoplasm.

Figs. 13, 14. Free parasites in leucocytes.

Figs. 15, 16. Intracellular Koch's bodies.



Gladys Roberts, del.

Gladys Roberts, del.

THEILERIA TACHYGLOSSI.



# AN INVESTIGATION INTO THE CAUSES OF THE PREVALENCE OF ENTERIC FEVER IN KINGSTON, JAMAICA ; WITH SPECIAL REFERENCE TO THE QUESTION OF UNRECOGNISED CARRIERS

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## TEN CHARTS

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## I. INTRODUCTORY

The extensive prevalence of enteric fever in the town of Kingston, Jamaica, and its suburbs has been for some years a cause of considerable uneasiness, amounting at times to actual alarm. This uneasiness is fully justified when it is considered that the city is ideally situated in that it is exposed to bright sunshine practically all day long and on nearly every day of the year, for the weather might be regarded as that of perpetual spring and summer with occasional showers. The disposition of the town is that of a gentle slope from the upper parts right down to the sea, and therefore with an ideal natural drainage. The obvious inference is that the reason of the undue prevalence of enteric fever must be looked for in the inhabitants themselves. Part of the town is provided with a water-carriage system of sewage disposal, but a large part is not. This part reminds one very much of the old time military camp, when each fresh company of troops arriving, encamped upon or close by the latrines of the last, or, when the troops were stationed in a place for some time (comparable to the stationary population of Kingston), they were from a sanitation point of view living on their own dunghill.

Chart I, compiled from figures which have been given to me by the kindness of the Acting Medical Officer of Health, shows that the number of notifications of typhoid fever begins to increase in March and reaches a maximum in June. A considerable proportion of these, of course, are notified by medical practitioners in the city without any bacteriological examinations, and consequently include many which are perhaps not enteric fever at all. I think this inference is warranted, because if the figures are compared with those from the laboratory, where specimens which are sent up from suspected cases are examined (see Table I), it will be seen that as a rule more than half those examined give negative results. We may, therefore, take it that a certain fairly large proportion of those notified as typhoid fever are not true cases of the disease. I consider that I am further justified in saying that the laboratory figures, although based on a smaller total, are more likely to be accurate as regards the disease in question than those given by the Medical Officer of Health as 'notifications.' This must not be

implied as reflecting in any way on the diagnostic capabilities of the medical practitioners here, but it is an undoubted fact that many cases which exhibit a continued fever of obscure nature, though not enteric, are diagnosed as such when no bacterial tests—Widal, culture of the blood, faeces, urine, etc.—are carried out. Hence, although it is probable that few cases of true clinical enteric fever are missed, the number notified would show an excess in the actual prevalence of the disease in the clinical sense. This remark applies generally to all towns. In hospital records, such as I have more

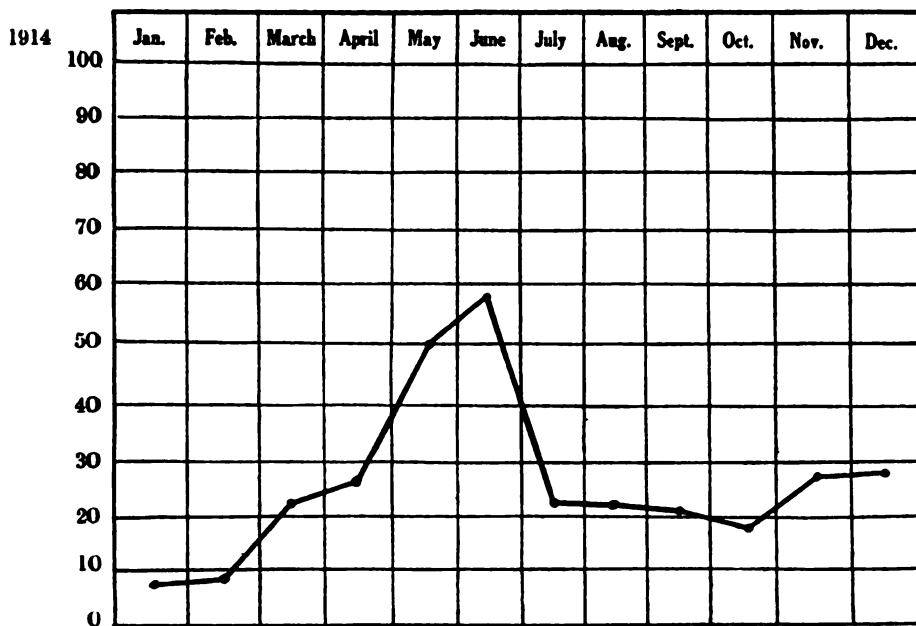


CHART I.—Notifications of Enteric Fever cases in Kingston during 1914.

particularly to deal with, a different state of things may exist. Having a pathological laboratory in the centre of the town, any doubtful case is subjected to a blood examination, and thereby two errors may be rectified.

Firstly, by this means patients with obscure febrile symptoms, but not clinically indicative of enteric fever, might have to be reported as such, owing to positive laboratory findings.

Secondly, suspected cases, which would be reported as enteric fever because of this suspicion, are not so notified owing to negative

results of laboratory tests, or the notification is delayed till other symptoms render the diagnosis clear.

Although these two points are antagonistic, the first tending to increase while the second would lead to a diminution of cases notified, the latter certainly preponderates, as is shown by the appended Table I, where it will be seen that of the specimens sent up some 50 per cent. proved to be negative. In other words, about one-half of suspected cases are not suffering from enteric fever, that is, the notifications, as stated above, are in excess of the actual prevalence of the disease. Cases wrongly diagnosed as enteric fever will, it is true, erroneously swell the notifications, but if laboratory examinations of such are made free (see p. 269), these diagnoses can be rectified later, and the Medical Officer of Health can make the necessary alterations in his records.

One of the main dangers of a wrong diagnosis is that such cases may then be traced to the operation of some factor in the spread of typhoid infection which really has played no part, the case not being one of typhoid fever at all. On the other hand, this is a very small point in comparison with the overlooking of positive cases. In other words, the erroneous diagnosis of a non-enteric case as enteric is venial compared with the failure to diagnose a true case because the clinical manifestations happen to be anomalous, through omitting to make use of a laboratory when it is situated centrally, and thus allowing a possible carrier to go about unwarned.

It must be remembered that among the 'negative' cases are included some which on subsequent testing yielded positive results, since some specimens are sent up too early in the disease (second or third day, perhaps), while in others agglutinin formation may be delayed.

The taking of a sample of blood for a Widal test is simple and causes practically no pain, but in the interpretation of the result there are certain pitfalls. These have been dealt with in more detail elsewhere (Scott, 1913), so it will suffice merely to mention the following facts:—

- (1) Though a positive result is obtained in the majority of cases (95 per cent.) at some time or other of the disease, there is, it would appear, a small percentage in whom it is not given. Into the reasons put forward for this I need



TABLE I.—Showing the numbers of specimens sent to the laboratory month by month for Widal's test during the year 1914, with the results.

Month	Number sent	Positive	Percentage positive	Negative	Percentage negative	Doubtful	Percentage doubtful
January .....	21	7	33	13	62	1	5
February ...	26	8	31	17	65	1	4
March .....	54	23	42	29	54	2	4
April .....	68	27	40	39	57	2	3
May .....	119	40	33	75	63	4	4
June.....	109	59	54	47	43	3	3
July .....	62	22	35	39	63	1	2
August .....	65	22	34	40	61	3	5
September ...	69	21	30	46	66	3	4
October .....	71	19	27	51	70	2	3
November ...	71	24	34	43	61	4	5
December ...	73	21	29	49	67	3	4

not enter now—deficient agglutinin formation, excess of agglutinoids, etc.

- (2) That a positive result is in many cases, one might almost say in most, not obtained until the end of the first week of the illness, or even later.
- (3) That in cases of exceptional severity the test may give a negative result.
- (4) That a positive result does not necessarily mean that the present illness of the patient is enteric fever. Patients who have been through an attack previously, it may be years before, not uncommonly give agglutination, especially if they are carriers, but by no means always so.

Blood culture, of course, is best for early diagnosis. The percentage of cases from which the organism can be isolated diminishes from 96 in the first week to 35 in the fourth; that is, the value of the Widal test gains as that of the blood cultivation diminishes.

To state briefly the effects of the Widal results on the notification returns (in addition to what was stated before): negative results, owing to early stage of disease, delayed reaction, and so forth, would tend to diminish the number notified, while positive results occurring in old, cured cases, suffering now from totally different affection, would tend unduly to swell the returns.

Bearing these few facts and reservations in mind, the charts giving the curves of notifications to the Medical Officer of Health for this disease for the last seven years are most instructive (see Charts II—X, pp. 279-284).

In nearly all of these charts it will be noticed that there is a distinct rise in the number of cases notified in the second quarter of the year, and in some years a small increase during November. In 1912 the November increase was markedly high (see Chart VI), the reason for which I am unable to conjecture at this interval of time. Comparing the average table of notifications (Chart X) or the 1914 one (Chart VIII) with the laboratory results for the same year (Table I), the first-named increase is most distinct.

The epidemiological aspect of the question is a fascinating one, but appertains rather to the domain of the Medical Officer of

Health, and I must pass on to the points which belong more to my own investigations.

Chart X reveals the startling fact that for the past seven years—I have not been able to obtain reliable statistics prior to that—the average number of fresh notifications of enteric fever cases has never fallen as low as 15 in any month of the year, while in May and June the average for the same period reaches the high figure of 30 and over.

Briefly stated, then, the problem to be solved is two-fold, namely:—

- (1) Why is the permanent enteric fever prevalence so high in a town favourably circumstanced as regards sunlight and natural drainage?
- (2) Why is the prevalence at its highest in the late spring and early summer months of the year?

I am strongly of opinion that the solution of the problem of enteric fever prevalence, and thereby the hastening of the desideratum of the final suppression of the disease, is more likely to be reached by studies of the affection in areas and places where it is endemic than by investigations of local acute outbreaks where of necessity the investigation has to be, to a certain extent at least, a hurried one. A town in which the average notification total in the best months is between fifteen and twenty cannot be regarded otherwise than as an endemic area.

In beginning an investigation into the causes of such prevalence of enteric fever in Kingston, one's thoughts were naturally first directed to the usually recognised sources of the spread of the disease, namely, food and water, flies, and dust.

## II. WATER SUPPLIES

Bacterial examinations of the Kingston water supplies are regularly carried out at this laboratory in order to safeguard their purity. The supply is three-fold; of these, two are in constant use, while the third is a subsidiary supply called into requisition in dry months, when the others prove inadequate for the demands of the population. If the analyses at any time give indications of deterioration from the usual standard, which is a good one and well

up to that recognised for tropical waters, that is, if any indications of excretal contamination are found, the fact is immediately reported to the Central Board of Health and to the Kingston General Commissioners who control the supplies, and steps are at once taken to locate and remedy the mischief.

It came as a matter of great surprise, therefore, in June, 1914, when the town was scared by a report from one in authority in public health matters here, that the water supply, and especially the auxiliary one installed comparatively recently at great expense and after several careful analyses, consisted of 'diluted sewage,' and could 'by no methods be rendered fit for drinking,' and was the cause of the large incidence of enteric fever.

Fortunately, to put the matter briefly, the report proved to be quite unfounded, a mere canard, for the incriminated source of supply was not, nor had it been for some time, in use, the other two sources proving quite sufficient for the demands of the population. Moreover, these latter showed no deviation from their customary standards.

To discuss in detail the points brought forward would unduly prolong this paper, so I will content myself with stating that against the supposition of the disease in Kingston being water-borne are the following facts :

1. The population of Kingston is estimated at 58,352, and the notifications of cases of enteric fever include all those in the Public General Hospital, which draws not only from Kingston, but also from the neighbouring parish of St. Andrew, at all events the lowest and most densely populated part of it. At the very smallest computation, therefore, we may take it that the combined population from which the notifications came was 60,000.

During May, the month in which the greatest number of cases was notified, there were fifty notifications, that is, less than 1 per 1,000. This is a large proportion in a town with every natural advantage for drainage, but a very small proportion as compared with what would occur if the infection were water-borne, since over 58,000 individuals drink this water. The records of the Worthing outbreak in 1893 give 557 cases in three weeks in a population of 15,000, or approximately 37 per 1,000. In the Maidstone epidemic of 1897 there were 1,201 cases in three weeks out of a population of

33,830, or 35 cases per 1,000. In Lincoln in 1905 in the same period approximately 11 per 1,000 of the population were attacked. In Kingston, as already stated, it is less than 1 per 1,000.

2. The penitentiary, with a large number of inmates, and the lunatic asylum of 1,400 patients, use the same water, and moreover, water from no other source, yet amongst these during the same period there were no notifications at all.

3. The different quarters of the town, though having the same water supply, contributed in very different proportions to the total of cases of enteric fever notified. Thus, in the month referred to, from the N.E., N.W., S.W. and S.E. districts there were notified respectively 11, 23, 7, and 5 cases, that is, 34 in the northern to 12 in the southern parts.

In subsequent months for which I have, by the kindness of Dr. Crosswell, the Acting Medical Officer of Health, been able to obtain details, in July, out of a total of 19 for all four districts, 15 came from the northern and 4 from the southern; in August, 19 out of 28 were notified from the same parts; in September, 12 out of 20; in October, 11 out of a total of 17; in November, 12 out of 17. The significance of these facts will appear later.

### III. MILK AND OTHER FORMS OF FOOD

Personally, I have not observed any cases in Kingston in which the infection could be definitely traced to contaminated milk supply. This is a matter for the Medical Officer of Health, and is outside my particular province. I am on sure ground, however, in stating that here, as in most tropical countries, milk is very carelessly handled, and the cleanliness of the vendors and their receptacles is by no means above suspicion. Watering the milk is a common practice, and if the tap is too far off, a nearer source is called into requisition without compunction. One vendor has been seen to abstract milk and fill up to the original bulk with liquid flowing down the gutter at the side of the road. When it is remembered that these same gutters constitute for the poorer inhabitants the State-provided latrine, one source of infection is not far to seek.

Besides the faulty methods of handling and delivering milk, another and more remote source of infection is possible. Few, if

any, of the purveyors—I personally know of none, except possibly at the Government farm—make even a pretence of sterilising milk bottles or cans. So infection of milk may arise by washing the various utensils with polluted water, by employment of carriers, or of persons suffering from mild or ambulant forms of the disease.

As regards other articles of food, shell-fish is not eaten to any extent in Kingston, and certainly not by the poorer classes. Vegetables are brought into the town from neighbouring districts by small cultivators, who deposit their excreta close to the huts in which they live; they form a possible but not a proved source.

Sweets, cakes, and such like, are on sale at various dusty street corners, and it is only recently that the vendors have been ordered to keep these delicacies (?) under cover to protect them from dust, flies, or the fingers of intending purchasers. These people commonly take up one article after another before coming to a decision as to which gives most value for their money. The hovels in which sweets and foodstuffs are prepared are anything but above suspicion. Indeed, I know of one case where the sweets thus sold were actually being made in a hut in which an enteric patient was lying. This was only discovered by the merest chance, and the tracing of infection to its source when spread from unrecognised or unreported cases must of necessity often be a matter of chance. Nevertheless, it is clear that from the standpoint of preventive medicine, the unrecognised cases are of greater importance than cases which are erroneously reported as enteric fever.

#### IV. FLIES

These insects are very troublesome in some parts of the city, particularly in the poorer parts whence many of the enteric cases are notified. It is well known, in fact almost obvious, that the chances of dissemination of infection by the agency of flies in a well-sewered city are much less than in cases where proper care is not taken in the disposal of excreta.

In Kingston the parts unconnected with the water-carriage sewage system are those in which the majority of cases arise. In these quarters flies are troublesome at most seasons of the year, but the time when they become a positive pest is that of the 'Mango

season,' starting about May, and this is the time at which the enteric fever notifications begin especially to increase. This part of the question is barely touched upon here; I hope to consider it subsequently.

A fly census in different parts of the town, and the establishment of a correlation between this and the districts whence notifications of enteric fever are sent, would be an interesting matter, but is more within the province of the Medical Officer of Health than that of myself. With his consent, I would like to undertake such an investigation at some future period, if my other duties will permit.

Dust, though often very troublesome in Kingston, is not regarded as so potent a cause of the spread of enteric fever as has been believed, especially in a tropical country with prolonged exposure to the sun's rays. In fact, it would be more correct to say that as a direct cause dust accounts for very few cases, and that many of those formerly attributed to dust, as air-borne, may be more reasonably ascribed to the agency of flies.

House-flies have been captured in dwellings near badly-kept privies used by enteric fever patients, and the bacilli have been isolated from them. Experimental investigations (Hamilton, 1903) have shown that living bacilli may remain in or on the bodies of flies for twenty-three days after infection.

Cockroaches also, which, like the poor, are always with us in Jamaica, swarm near badly protected food, and may easily act as mechanical carriers of infection. Food protection, except from the point of view of larceny, does not enter into the domestic arrangements of the poorer inhabitants of the city of Kingston.

## V. SEWAGE DISPOSAL

A few words will suffice to sum up this section. The water-carriage system is laid down for the lower part of the town only. The upper parts, N.E. and N.W. districts, are largely furnished—more or less badly—with privy middens, dry earth closets, and so forth, nothing less than a standing and open invitation to flies, which freely avail themselves of the opportunity.

Some of the huts inhabited by natives in Kingston have the luxury of a privy to themselves. In other instances there is one to a

yard in which there may be four or more huts and as many families, living as one small related community—very much related—so that privacy is more honoured in the breach than in the observance. The gutter passing the front doors is so handy that it is by no means an uncommon thing to see children, and even adults, obey the calls of nature in the open street. Again, natives come down from the inland districts, walk 15 miles or more to bring produce to sell in the town, stay the night in Kingston or on the borders of the city, and sleep on the pavement at the side of the road. These people cannot be blamed for depositing their excreta in the road or in the gutter, for public latrines are not provided for them.

These itinerant vendors may account for a few cases, but if so the association would be practically impossible to trace. I think that in Kingston, considering the class of patients dealt with in hospital, with whom my special investigations shortly to be related were made, and the quarters of the town from which they came, there is but little importation of the disease from outside. It must arise, therefore, to a large extent, if not entirely, in factors whose operation is localised.

It has been shown from observations (by Rosenau, Lumsden, Kastle, and Anderson, 1909-11) extending over several years in the district of Columbia, U.S.A., that there is in general 'no striking difference in the prevalence of typhoid fever in the sewered and non-sewered districts,' but at certain seasons, particularly the time when flies are numerous, the non-sewered districts showed a much greater incidence.

Also, the chances of infection being conveyed to the healthy from close association with the sick are found to be considerably greater amongst those who dwell in residences provided with privies, than amongst those whose residences have a water-carriage system connected with the regular sewage system of the city.

In Kingston many of the privies are in a parlous state and far below the modern standard of a sanitary privy, if such there be. They are not water-tight, they are not protected from flies, even in the better houses they are often situated close to the kitchens and even dining and sleeping rooms. The kitchens are not screened, and flies can travel readily from the privy to the food. Also, as already stated, in the poorer quarters one privy may have to serve



for several families, so that many individuals may become infected from a single carrier or a common source.

There is always a danger, in investigating for one single cause, of ignoring or forgetting other contributing causes. Thus, an investigation at one time might elicit evidence pointing strongly to one factor, e.g., flies, while at another the evidence might be to the incrimination of some article of diet—milk, shell-fish, etc.—and tend to minimise the importance of the former. But a high general typhoid rate with increased prevalence at some particular season of the year makes one think firstly what general and continuously-acting factor is at work; and secondly, what factor or factors are more strongly operative at the time of greater prevalence, and whether the reason is to be discovered in an increased scattering of seed, increased receptivity of soil, or both combined.

In Kingston, where the variations of temperature are comparatively small in the different seasons and where perpetual summer reigns, the following factors must be considered: prevalence of flies at certain times, diminution of rainfall and concentration of pollution, or sudden heavy rains washing pollution from the banks of a stream into the supply, the ingestion of raw fruits, and the prevalence of other intestinal affections rendering the subjects more liable to contract typhoid fever when exposed to infection, and so forth.

The water supply, the fly nuisance, the sewage arrangements, are matters for the Medical Officer of Health to consider and deal with. I could not interfere with them without his full consent and co-operation. But even granting that these were unsatisfactory, there remains still unaccounted for the source whence the flies, or the food, or the dust obtained the organisms of the disease, and carriers are naturally suspected.

## VI. CARRIERS

The question of carriers is a matter with which my work as Government Bacteriologist is closely connected, but there were certain difficulties in the way of investigation to avoid interference with the work of the Medical Officer of Health.

There are difficulties also as to obtaining material, for persons in

health cannot be prevailed upon to send excreta in order that it may be determined whether or no they are typhoid carriers. The objection is quite a natural one, and more particularly would this be the case when the finding would lead to certain rules and restrictions being imposed. Moreover, it is fairly certain that, whatever rules were given and however mild the restrictions, they would be evaded in some way or other. A rule without an enforceable penalty for disobedience is better left unmade. The native who would be found to be so altruistic as voluntarily to place restrictions upon himself for the good of others would indeed be a 'rara avis.' Further, if the results of examination were negative, the case would by no means be proven, as the carrying might be intermittent.

The question of the percentage of enteric fever patients who become carriers has been studied by many investigators. The results, so far as the literature at my disposal goes, may be briefly given. Lentz (1905) states that about 4 per cent. of typhoid patients discharge the bacteria in their excreta at least ten weeks after convalescence. Schneider (quoted by Besançon) estimates the chronic carriers as 3 per cent. of patients. Lehmann and Neumann give 2 per cent. to 4 per cent. Nieter and Liefmann (1906-7) isolated 7 out of 250 patients (that is, 3 per cent.) in an asylum. Asylums generally appear to harbour an abnormally high proportion of carriers.

But cases excreting only for so short a time as ten weeks after the illness cannot be regarded as carriers in the real sense of the term. The percentage of chronic carriers is variously estimated at from 1·5 per cent. to 5·2 per cent. So far as I have been able to ascertain, the following figures are authentic:—

Out of 400 cases of enteric fever 6 excreted the bacilli for more than three months, giving a percentage of 1·5; of 316 cases 9 carriers, or 2·8 per cent., were found; of nearly 7,000 cases 166, or 2·4 per cent; while of the large number of 11,000 cases 411 carriers were found, a percentage of 3·7.

Altogether I have found results recorded of 18,431 patients, and of this large number 592 were detected as carriers, a proportion of 3·21 per cent. We shall be safe in taking it that, as an average, some 3 per cent. of patients become carriers for three months at least.

Of 1,800 healthy subjects reported upon by Kayser in 1907 giving no history of previous typhoid fever, 27, or 1·5 per cent., were found to be excreting the bacilli. In Columbia the excreta from 1,000 healthy persons were examined, and of these three were found to contain the organisms; one of these excreted them in the urine, the other two in the faeces; all three were males.

Returning to the condition of things in Kingston, my suspicions were that, as the enteric rate is so high, there might be individuals going about apparently in good health, who were unwittingly spreading the infection, or that the percentage of carriers here is greater than has been found to prevail in most places.

## VII. BACTERIOLOGICAL INVESTIGATIONS

It is now an established fact that during an attack of enteric fever the bile frequently becomes infected; this is corroborated by my results as shown in the table of cases so examined (see Table II).

If now, as is also known to occur, an inflammatory process arises in the gall-bladder, the bacilli may be discharged either continuously or at intervals from the bladder into the intestines for periods of almost indefinite duration.

Progressing a step further, it may be said that in many cases the chronic bacillus-carriers are individuals harbouring the typhoid bacilli in their gall-bladders.

I determined, therefore, to make cultivations from the bile of every patient dying in the general hospital—regardless of the actual cause of death as diagnosed clinically or discovered post-mortem—in order to find out whether any, and if so how many, were carrying the organisms in their gall-bladders at the time of death.

Of course, in a large percentage of patients admitted with a history of enteric fever and dying from this disease, and also in patients wrongly diagnosed, but actually suffering from enteric fever, one would naturally expect to find the organism. But there was yet another class for which I particularly wished to look out, namely, patients who were admitted into hospital for some affection totally unconnected, or not suspected of being connected with enteric fever, and who gave no history of any previous attack of the disease or of any prolonged fever which might be so explained. These persons,

by harbouring the *Bacillus typhosus* in their gall-bladders, had possibly, if not probably, been acting as carriers, and, had they chanced to recover, would have again gone about spreading the organisms broadcast. These were the individuals who constituted a danger to the community, and if such were found to exist, the fact would go far to account for the undue prevalence of enteric fever in Kingston.

It is but right to call attention to the fact that the mere denial on the part of the patient as to his having suffered previously from the disease does not exclude the possibility of his having passed through an attack. The native is so accustomed to short attacks of malarial fever that any 'fever' of a prolonged or severe character usually makes a permanent impression on his mind. When he speaks of a 'bad fever' lasting for two or three weeks, this is quite sufficient to enable it to be stated that the history evinces a strong probability of the patient having had an attack of enteric fever in the past.

The investigation into the results of bile cultivations is still being continued. Up to the present 120 cases have been dealt with, sufficient for a preliminary note, and sufficient to form a basis for discussion (see also Addendum, p. 269). Before entering more fully into a consideration of the various points which present themselves, I would beg to call attention to the appended list of cases where the results are briefly recorded (Table II, pp. 272-278).

The following points worthy of more detailed mention present themselves on a perusal of this table:

1. The number of cases exhibiting post-mortem the signs of enteric fever from which a positive result was obtained from cultivation of the bile, and arising out of this, the number of cases with similar signs from which the result was negative.
2. A corollary to the last, namely, a consideration of the cases which showed no signs of enteric fever, but from which a positive culture was obtained.

Other points of interest which declared themselves during the investigation were:—

1. Peculiarities of the isolated organism in certain instances.
2. Absence in most cases of macroscopic signs of inflammation of the gall-bladder.

(i) *The method of procedure employed in each case*

As soon after death as possible, before starting on the usual post-mortem routine, the gall-bladder was exposed, the surface was sterilised by searing, and the organ held by sterile forceps. A small incision was made with sterilised scissors. Tubes of peptone broth were ready containing quantities of a 1 in 10,000 solution of brilliant green, varying from 0.03 c.c. to 3 c.c., namely, 0.03, 0.06, 0.12, 0.18, 2, and 3 c.c., the brilliant green being added immediately before the tubes were taken to the post-mortem room. A loopful (4 mm. in diameter) of the bile was introduced into each tube. Six tubes were sometimes used, but on no occasion less than three, because it was early noticed that the brilliant green had a variable inhibiting power, not always proportionate to the amount added. In other words, some strains of the bacillus obtained showed less resistance to the brilliant green than did others.

The tubes after inoculation were placed in the incubator at 37°C. and examined on the following day. If there was but a slight growth with faint cloudiness of the medium, further examination was postponed for another twenty-four hours. If there was no growth by that time the tubes were kept for a week before the bile was noted down as being 'sterile.'

If, and when, growth occurred a loopful was plated on to Reibelagar for isolation, and suspicious colonies, non-lactose fermenters, were transferred to nutrient broth. Secondary tests were then carried out with various sugars, namely, glucose, saccharose, lactose, maltose, mannite, dulcitol, and sorbitol.

Any organism isolated, which gave the usual typhoid reactions, was then tested for its agglutination power with an immune serum which I prepared, and which gave an agglutination titre of 1 in 4,000 with its own producing strain. Only those organisms which gave a positive result with the serum diluted to about this degree were regarded as true *Bacillus typhoides*. As is well known, to test with the serum of a typhoid patient or convalescent is quite inadmissible for the identification of a suspected bacillus. In such a serum besides the main specific agglutinins for the *Bacillus typhoides*, there may be present a whole series of group-agglutinins, agglutinoids and iso-agglutinins, in such concentration as to constitute a source of gross errors.

In the investigation, control tests were carried out with corresponding dilutions of normal serum of the derivative animal, to exclude the chance that the agglutination observed with the immune serum might be an action of a normal serum on the strain of bacterium under examination.

A second control was also put up, using normal saline in place of the serum, in order to exclude any incidental agglutinating action of the diluent—a pseudo-agglutination—which might appear from spontaneous clumping or insufficient emulsification.

Bruns and Kayser (1903) propose to use only a low dilution, such as 1 in 100 of the immune serum, but this course is liable to lead to erroneous conclusions, which need not be fully discussed here. A long series of dilutions has been made in each of my cases in order to determine in what degree of dilution of the serum the agglutination would take place. As Pfeiffer, Lipschütz (1904), and others have pointed out, only when this corresponds fairly approximately with the estimated titre of the serum ought the agglutination to be regarded as evincing the true typhosus nature of the organism in question.

(ii) *Cases with post-mortem signs of enteric fever from which a positive result was obtained on cultivation of the bile.*

Out of the one hundred and twenty cases, twenty-eight were shown at the autopsy to be suffering from enteric fever, that is to say, definite macroscopic lesions of the disease were present. In twenty-four of these the *Bacillus typhosus* was isolated from the bile, and in one other, whose blood during life had given a positive agglutination with *B. paratyphosus* A, and negative with *B. typhosus*, and who presented symptoms typical of enteric fever, the *B. paratyphosus* A was isolated. We may therefore say that from twenty-eight cases of enteric fever the causative organism was isolated from the bile in twenty-five, or in 89·28 per cent., although I am aware that the reckoning as a percentage with so few cases as twenty-eight is liable to error. This percentage number agrees almost exactly with the figures of Forster and Kayser (quoted by Hewlett), who obtained pure cultures from the gall-bladders of seven out of eight cases. A better comparison may be made between these cases and

mine if we divide my twenty-eight into series of eight. For the first eight I obtained the *B. typhosus* in pure culture six times, and twice the organism was associated with a lactose-fermenting one, which proved to be the ordinary *Bacillus coli*. The *B. typhosus* was thus isolated in all the first eight. One of the cases in which the *B. coli* was present also died from general peritonitis following perforation of a typhoid ulcer, and the bile may have become infected by general systemic infection, or, what is an equally probable explanation, the *B. coli* may have entered as a contamination in making the culture. If the former explanation is the correct one, the fact shows that the bile and brilliant green do not always have an inhibiting action on *Coli* organisms.

With regard to the second series of eight, I obtained the *B. typhosus* in pure culture in six, and *B. paratyphosus* A in another, also in pure culture; in other words, the organisms were present in pure culture in seven out of eight. In the third series, *B. typhosus* was obtained in pure culture in six, one remained sterile, while from the other *B. coli* only was obtained.

The remaining four of the twenty-eight yielded the *B. typhosus* alone in each case.

Hiss and Zinsser (1910) in their text-book merely state that the 'typhoid bacilli have been frequently observed in the gall-bladder at the autopsy.' They do not mention how frequently. According to Meyerstein (1907) the bile of typhoid patients nearly always contains the *Bacillus typhosus*, though its presence does not often give rise to clinical symptoms. Its presence may, however, set up inflammatory processes in the wall of the gall-bladder. Thus Stewart (1901) found a condition of cholecystitis in about 1 per cent. of cases. Similar findings have been reported by Forster and Kayser (1905), Hilgermann (1909), Findlay and Buchanan (1906), Marmann (1908), Nieter (1906-7), and others.

The presence of the bacilli in the gall-bladder of patients suffering from enteric fever is not to be wondered at when we consider that the disease is, in the early stages at least, a bacteriaemia; that experiment has shown that it is by way of the blood-stream that the gall-bladder becomes infected, and that the conditions prevailing there appear to have a favourable influence upon the growth of this organism. For it has been shown by

Blackstein and Welch (1891) that after intravenous injection of the bacilli into guinea-pigs they were recognisable in the bile four months later, when all other organs appeared to be completely free from them, while after subcutaneous, intraperitoneal, and intra-gastric inoculation the bile remained sterile, though it was infected after intravenous injection, even with the cystic duct ligatured (Doerr, 1905). This appears to be the usual mode of infection of the bile in man, namely, by way of the circulation. It is only fair, however, to mention that Koch and Chiarolanza (1909) have held that there is an alternative route direct from the intestine to the bile through the ductus choledochus, at all events in experimental infection of rabbits. In the case of the human subject, however, this latter is very unlikely, seeing that the lower part of the small intestine is that most affected, and it would indeed, with respect to the local condition, be a 'long way to go' when the route via the blood-stream is so much more accessible and rapid. Moreover, the bile appears to be infected so very early in the disease.

- (iii) *Cases which showed no post-mortem evidence of enteric fever, and in which no history of such was obtained, but which, nevertheless, yielded a positive result on cultivation of the bile.*

This group is of the greatest importance in lending support to the suspicion on which the investigation was undertaken, namely, that unrecognised possible carriers are going about in Kingston in larger proportions than have been estimated in other countries.

As has been already stated, some 3 per cent. of patients become carriers for a considerable time. Of this first series of one hundred and twenty autopsies there have been found four who up to the time of onset of their final illness had been going about apparently in perfect health, who gave no history of having had an attack of typhoid fever. In one case, No. 2, there was an indefinite history of prolonged fever which might possibly have been typhoid, though from the symptoms and post-mortem signs this might equally well be ascribed to tuberculosis. The four mixed freely with their fellows, and lived in the poorer, insanitary, and unsewered parts of the city.



These cases are worth quoting briefly :

1. C. C., male, aged 49 years, white, No. 7 in the appended list, Table II. Admitted to hospital 4 p.m. March 31, 1914, with a history of having at 9 a.m. 'taken rat-poison in mistake for phenacetin.' He died at 11.40 p.m. The fact that more than 400 grains of arsenious acid were found in the stomach and viscera, even after the vomiting which had continued for ten hours, points to deliberate suicide, for no one would take nearly an ounce of phenacetin for a single dose. His neighbours stated that he went in and out amongst them apparently in perfect health till the day of his death. In bile culture *B. typhosus* was obtained.

2. B., a Coolie woman, aged 21 years, No. 34 in the list, when admitted complaining of 'fever, cough, and pain in the right side of the chest.' Expiration was prolonged, and there were rhonchi audible over both lungs.

The diagnosis of phthisis was made, and the patient died early the following morning.

At the autopsy the lungs showed minute scattered nodules with caseous contents, possibly tuberculous, but the chief finding of interest was that the gall-bladder had practically disappeared, and there was an ulceration into the duodenum just beyond the pylorus, with a gall-stone about the size of a small hazel nut in the aperture.

There were three abscesses in the liver, one cavity being the size of a tangerine orange.

From the nucleus of the gall-stone, the only one found, a pure culture of *B. typhosus* was obtained.

3. S. D., male, aged 19 years, black, No. 73 in the list, was admitted with a history of six days' 'cough, pain in the right side of the chest, and fever.' The percussion note over the right lung, lower lobe, was quite dull. 'No breath sounds could be heard, but vocal fremitus and resonance increased.' Widal reaction was negative. The patient died ten days later.

At the post-mortem the right lung was quite solid; the upper and middle lobes in a state of grey hepatisation, the lower lobe in a condition of purulent infiltration, and in one place broken down to abscess formation. The lung was large and heavy, and the liver so displaced that the upper margin only reached one and a half fingers'-breadth above the costal edge.

This, then, was a case of unresolved lobar pneumonia. No history whatever was obtained pointing to enteric fever, but a culture of *B. typhosus* was yielded by the bile.

4. E. R., female, aged 21 years, black, No. 113 in list, was admitted to hospital on October 26th, 1914, with dyspnoea and general oedema, especially of the lower limbs. The urine was albuminous and contained granular and hyaline casts. Death took place on the following day.

The autopsy revealed a condition of chronic nephritis, the renal capsules were adherent, the surface of the organs granular, the cortex narrowed to about half the normal ratio, and there were two small cysts in it. The heart was enlarged, the left ventricle being hypertrophied, and the weight of this organ when empty was 480 grams. There was no sign of enteric fever found, and no history obtained of any such disease. A pure culture of *B. typhosus* was isolated from the bile, which agglutinated after three subcultures with the typhoid immune serum in as high a dilution as 1 in 5,000.

Exclusive, therefore, of cases showing evidence of enteric fever at the autopsy, the bacillus has been isolated from the bile of four subjects:

1. Dying from arsenic poisoning, suicidal.
2. Dying from multiple abscesses of the liver.
3. Dying from pneumonia.
4. Dying from chronic nephritis and heart disease.

It would hardly be fair to draw conclusions from so few cases as one hundred and twenty, and the investigation is being continued (see Addendum, p. 269). All that can be said at present is that, apart from patients treated for enteric fever at the hospital, and apart from cases showing signs of this disease post-mortem, there have been three among the first one hundred and twenty who were harbouring the *Bacillus typhosus* in their gall-bladders at the time of death. In a fourth who had done so, the organism was found in a gall-stone which had ulcerated into the duodenum.

If we deduct the number of those who were suffering from the disease, showing definite signs of it at the autopsy, we may state that out of ninety-two subjects there were four who were harbouring the organism of enteric fever.

(iv) *The deductions which may be drawn from the fact of isolation of the bacillus from the gall-bladder or its contents*

1. The subjects are chronic typhoid carriers. This is possible, but could not be regarded as proved unless repeated examinations of the excreta yielded positive results. In my cases this was impossible, as they did not come under my observation till after death.

2. The subjects are temporary carriers.

(i) They may, in the absence of history, have passed through an attack of enteric fever and forgotten or not have known the fact, either because of the mildness of the infection, or because of time elapsing, or because the illness had been wrongly diagnosed. The following cases show that the last may certainly occur :

No. 4, diagnosed lobar pneumonia.

No. 5, diagnosed pneumonia.

No. 9, diagnosed pneumonia.

In these three, typhoid ulceration was found but no signs of pneumonia.

No. 10, diagnosed general peritonitis. This was true, but the peritonitis arose from the perforation of an enteric ulcer which had not been suspected.

No. 88, diagnosed cerebral haemorrhage. There were the usual signs of enteric fever, but none whatever of cerebral haemorrhage.

No. 99, diagnosed pneumonia. This was present, but there had been no suspicion of enteric fever, which was evident with its typical pathological changes.

(ii) They may be 'porteurs sains' in the sense of never having had the disease. In this connection I may quote an interesting case which came to my notice as Government Bacteriologist more than a year ago. It was mentioned in my paper already referred to, and published in the 'Practitioner' for November, 1913 :

'A child of nine months suffered from an attack of typhoid fever, proved by isolation of the bacillus. The question then arose as to the method by which the child could have become infected. The little patient's mother was the nurse in charge of the enteric ward at a general hospital in the island, and it was thought that she might possibly have taken some milk or other food from the ward home to the child. A specimen of the mother's blood was asked for, and it was found to give a very marked agglutination of bacillus typhosus in high dilution.

'The nurse was perfectly certain that she had never suffered from typhoid fever, or, in fact, from a prolonged fever of any kind; she had always been healthy, and "never remembered being ill in her life" (to quote her own words). The next step was the examination of her stools and urine, and the bacillus typhosus was isolated from the latter; in short, this nurse was definitely a carrier, though never having herself suffered from the disease, and she seems without doubt to have conveyed the disease to her child.'

If the subjects are 'porteurs sains' in the sense mentioned above, that is, the organisms are present without setting up the disease, we may find here support for the X Y Z theory of Pettenkofer. Thus, it is possible that the bacilli may be harboured in certain situations (in my cases the gall-bladder) for a lengthy period, but until the other components of the aetiological complex are superadded no disease results. We may, perhaps, compare it to the lowering of resistance to the *Bacillus tuberculosis*, allowing development of the organism and production of the disease.

Against this in my cases is the fact that two, possibly three, of the subjects were considerably debilitated by illness, and there were, nevertheless, no signs of enteric fever supervening. This argument, it must be confessed, is of the nature of a two-edged sword, for, firstly, mere debility by any illness may not be the Y or Z of the aetiological complex, but some element more specific in nature may be necessary. Secondly, some of the cases which showed signs of enteric fever post-mortem, although a different diagnosis had been made clinically, might be instances of the supervention of enteric on a previous debilitating disease. This, again, opens up the interesting question as to whether cases of enteric fever arising in patients who have been in hospital for some time, a month perhaps, may not in some instances be of 'spontaneous' or 'autogenous' origin, instead of being regarded as cases of 'contraction of disease in hospital.' By the terms spontaneous or autogenous I imply the 'porteurs sains' who develop the disease after some other debilitating illness, for example, typhoid fever following dysentery, and not merely ordinary cases with a prolonged incubation period.

(v) *Peculiarities of the organism isolated in some instances.*

1. *Motility.* As has been noted by Fischer (1909), I have found several times that when freshly isolated from the body, that is, from the bile in my cases, the bacilli were but slightly motile, and in some

instances were devoid of motility altogether. But he goes on to say that even after prolonged subculture the motility was only slight. This has not been my experience. After the third or fourth transference to nutrient broth the normal motility was always regained; or by transference from agar to broth alternately.

I have not so far had to employ any of the special media for this purpose, such as Lösener's serum, Terni's peptone-free glycerin bouillon, or the 2 per cent. glucose broth of Germano and Manrea.

2. *Agglutinability*. In my cases I have not yet met with an organism which, while giving the cultural tests and sugar reactions of the *B. typhosus*, was completely non-agglutinable with the prepared immune serum. In several instances, however, the first subculture showed very slight agglutinability, but, after subculture to the third or fourth generation and subsequently, the organisms proved normally agglutinable.

In two cases I found a mixture of two typhosus colonies, one easily agglutinable, the other only agglutinating with difficulty, occurring from the same subject. A further point was also observable, namely, that the former—the one readily agglutinable—showed marked motility, while the latter—the poorly agglutinating strain—was practically non-motile. Sub-cultivation, however, on ordinary agar and in broth alternately, produced organisms equally motile and equally agglutinable in the two cases. Thus, it is seen that the diminution or absence of motility and a low degree of agglutinability of a newly isolated strain of the organisms go hand in hand. By further observations it was found in carrying out the agglutination reaction that time was an important factor in some cases. Thus, a strain which proved to be agglutinated to a slight degree only within the ordinary time limit of two hours, would in some cases, not in all, be strongly agglutinated if left for several hours—up to 24—at room temperature, less if kept in the incubator at 37° C.

Two of the explanations, which have been put forward to account for the low degree of agglutinability of typhoid bacilli freshly isolated from the body, are worthy of being mentioned in greater detail; namely, that of Bail (1901), Müller (1903), Kirstein (1904), and others, and that of Nicolle and Trenel (1902).

Their findings, some of which I have been able to confirm, may be briefly stated thus:—

A culture of typhoid bacilli agglutinable in high dilution of the immune serum was inoculated intraperitoneally into a guinea-pig. After remaining a few hours the agglutination test was again applied, and the titre of agglutination was reduced from the previous 1 in 4,000 almost to nil; there was incomplete clumping even with 1 in 300. This has been explained by supposing that the bacteria which have been inoculated seize upon the agglutinophores which possess the haptophore groups but not the zymophore, and thus put them out of action. Bail (1901) similarly explains the reduction of agglutinability of bacilli growing in a diluted immune serum, for the normal degree of agglutinability is restored after a few transferences back to ordinary nutrient media. It is but right to state, however, that Müller (1903), Hamburger, and others regard the latter phenomenon rather as a definite attempt at immunisation of the bacillus against the injurious action of the immune serum, though they are all agreed as to the subsequent restoration to normal agglutinability. It must be noted that this reduction is not marked in all strains. We may thus briefly state this view: Under certain conditions typhoid bacilli present in a patient seize upon the agglutinoids and so lead to the production of cultures of the organism which are but poorly agglutinable.

The second explanation rests upon the findings of Nicolle and Ternel (1902) that cultures of the *Bacillus typhosus*, normally motile and readily agglutinable, lost these properties when grown at 42° C., though they regained them on subsequent cultivation at 36° C. They inferred that the high temperatures which some typhoid patients exhibit might produce a similar change in the bacilli, reducing their agglutinability. Higher temperatures still, such as 50° C., on the other hand, appear to bring about a hastening of the agglutination and to render it more complete.

Eisenberg and Volk (1902) have shown by their experiments that the non-agglutinable or poorly agglutinable strains may be, nevertheless, identified by means of the immune serum. Heating the cultures of typhoid bacilli or treating them with weak acids deprived them of their agglutinability. Bacilli so treated are capable of binding large amounts of agglutinin, hence the inference that such treatment renders nugatory that part of the agglutinable substance which is actively concerned in agglutination. The haptophore group, however, is still present, for it is found that if such altered

bacilli be brought into contact with a typhoid immune serum all the typhoid agglutinin can be removed, so that it will no longer agglutinate any typhoid bacilli. This, as Wassermann has suggested, might be usefully employed for the identification of typhoid bacilli in those cases in which there is a suspicion that the organisms have, owing to extraneous influences, lost their normal agglutinability. But, seeing that cultivation again on ordinary nutrient media soon restores the normal agglutinability, this method of Wassermann would, or need only, find application where a speedy diagnosis is urgently called for.

(vi) *Length of stay of the organism in the gall-bladder.*

As regards the length of time during which the bacillus can exist in the gall-bladder, the limit has not yet been fixed. Cases of 16, 17, and 20 years have been reported by Zinsser (1908), Droba (1899), and Hunner-Writer (1905), respectively. I myself related the case of a faecal carrier of *Bacillus paratyphosus A* in 1911, whose primary attack occurred in 1889, twenty-two years previously. Cases reported by Dupré, Ramond, and Faitout, are quoted by Besançon, but it must be remembered that all these refer to the duration after an attack of the disease. The four mentioned in my table, Nos. 7, 34, 73, and 113, gave no history of any previous attack at all, and the time in them is impossible to determine.

(vii) *Presence of the bacillus with and without obvious inflammatory changes in the gall-bladder.*

Doerr (1905) has stated that the bacilli make a prolonged stay in the gall-bladder only if an inflammatory condition of the mucous membrane is set up. In two of my four cases I could not make out any such condition. It is possible, therefore, that their histories were correct, that they had not suffered from the disease and that the gall-bladder was merely their temporary sojourning place, a port of call, as it were, in a 'porteur sain,' as the bacilli were on their way to being excreted. It may be incidentally noted that gall-stones are very rarely found post-mortem here. Of all the autopsies carried out by me during the last four years, I have only found them to be present in two instances.

(viii) *The question of atypical cases without characteristic changes.*

Some of those from whom the organism was isolated, although no symptoms were present and in whom there were no post-mortem sign of the disease, might, of course, be examples of the atypical cases without characteristic changes. Under this heading would come such as have been reported as cases of 'typhoid septicaemia'. If by this term we understand 'a systemic affection in which the causative organism not only gains entrance to the blood-stream but also multiplies therein,' I know of no recorded positive findings of such a thing in enteric fever, but an ordinary bacteriaemia is the normal condition at an early stage of the disease. Absence of evidence of intestinal affection, in spite of finding the bacilli in the blood, is less uncommon. Such may be explained by the fact that all transitions occur between the marked pathological changes in the intestines and a minimal, scarcely recognisable lesion, and this also apart altogether from the severity of the case. Also de Grandmaison (1900) and Krokiewicz (1908) have produced evidence that in several such cases definite formation of specific antibodies may be recognised in the serum. A second explanation is that mentioned in the last section, namely, that the bacilli have gained entrance into the body of a patient suffering from totally different disease, the subject acting the part of a passive carrier or receiver, as, for example, the entrance into the blood-stream of typhoid bacilli accidentally by way of a tuberculous enteritis, as in a case recorded by Busse (1908).

We must never lose sight of the fact that enteric fever is not an intestinal infection. It is, on the contrary, a general one with localisations of the bacteria in various situations, most frequently in the intestine, but often in the liver and biliary system, sometimes in the lungs, the bladder, the pleura, the meninges, and so on. Thus, there may be a considerable variety ranging between a general bacteriaemia without local infection specially marked in the intestines, and more definitely localised forms with symptoms of meningitis, broncho-pneumonia, cholecystitis, nephritis, arthritis, etc. Yet again the typhoid nature of the condition may be masked by accompanying infection, such as a streptococcal, or tuberculous one.



### VIII. PERSONAL CONTACT

With the histories often faulty, sometimes unintentionally, sometimes deliberately so, it is an almost impossible matter to trace the extent of personal contact and gauge how far it plays a part in spreading the disease in Kingston. The poorer inhabitants live often closely massed together, and many cases may thus arise from a common source of infection, as has been already stated.

When the large number of possible sources of infection furnished by typhoid carriers and by typhoid patients are considered, and the many ways in which infection may be conveyed from the diseased to the healthy, it can only be wondered why there is not more typhoid fever rather than why there is so much.

If the extensive prevalence of this disease is to be coped with in anything like an adequate manner, typhoid convalescents must be subjected to greater restrictions than they are at present. Particularly is this necessary with cases who pass cloudy urine (bacilluria). They must be restricted until their excreta have been examined bacteriologically and found to be negative. They must not be freed entirely from observation even then, because of the possible intermittency of the condition. As everyone knows, it is the usual custom to discontinue all supervision over enteric fever patients as soon as they are able to leave their beds. Consequently in the majority of cases (I know there are exceptions, for instance in the army, but otherwise the exceptions are rare) disinfection of stools and urine is stopped as soon as the convalescent is able to be up and about. To recognise how very unsafe this procedure is, we have only to call to mind the results of examination of the excreta of convalescents, and note the length of time during which many of them continue to harbour the organism in question.

Though it is of great importance and value to discover the main, or even one of the main, factors causing the spread of enteric fever in a district, particularly in a crowded town like Kingston, nevertheless, since the typhoid rate of incidence may be looked upon as a good sanitary index of the hygienic condition of a community, coincident improvements should be carried out in all the branches of sanitation, if the prevalence is to be materially reduced. The purity of the water supplies must be safeguarded, the sewerage

system should be modernised and the food supplies protected. The patients should be kept under observation for a more prolonged period than under present circumstances here (and so far as I am aware the same remark applies elsewhere). More care should be exercised both as to site for and manner of disposal of refuse. Measures should be taken to minimise the prevalence of flies. The community should be educated to greater personal cleanliness. In short, all the conditions of general sanitation should receive attention where such are found to be defective.

#### IX. LEGISLATIVE RECOMMENDATIONS

Having pointed out some of the various ways in which the undue prevalence of enteric fever may be accounted for in Kingston, it only remains for one to suggest means by which the possibility of spread may be to a large extent controlled. The following brief recommendations do not include the measures which have been already indicated, but apply rather to the legislative aspect of the question.

1. Rules should be made for early notification of all *suspicious* cases. Much valuable time is lost if the physician waits until he is practically certain, often a matter of a week and sometimes of a fortnight or more. The form of notification should be different from the usual one of 'In my opinion A.B. is suffering from enteric fever,' and might read thus: 'In my opinion A.B. shows evidence of symptoms which may indicate enteric fever in an early stage.' Seeing that the diagnostic ability of physicians is so variable, and that the symptom-complex regarded as pointing to enteric fever is so differently interpreted by individual medical men, the regulation might be made that any patient with unexplained fever of three days' duration should be so reported.
2. Blood cultures, if the case is in an early stage, or Widal's reaction after, say, the end of the first week should be carried out at the Pathological Laboratory free of charge to the patient.
3. Isolation of the patient should be required. In circumstances where this cannot be carried out at home to the

satisfaction of the health officer, removal to hospital with this end in view should be made compulsory.

4. If the patient is allowed to remain at his home, the health officer must be satisfied that adequate disinfection of the excreta not only can be, but is carried out.
5. No person continuing to live in the same dwelling as an enteric patient should be allowed to prepare, or manufacture foodstuffs (including beverages) without the permission of the health officer, given in writing.
6. Any room which has been occupied by an enteric fever patient must be properly disinfected before being again occupied.
7. Patients should remain under observation of the health officer until the excreta show on three consecutive examinations, at intervals of a week, absence of the causative organism.
8. All patients on discharge from hospital should be warned of the danger they constitute to others. Any infringement of the rules, as to abstaining from the preparation, etc., of food for others, must be made punishable.
9. Persons engaged in the manufacture, purveyance, or sale of food or drink, must, if called upon to do so by the health officer, furnish evidence that he or she is not a carrier. For this purpose he or she must allow examinations of the excreta to be made. If the results are negative a certificate is to be given to that effect which will hold good for a certain time, unless there are definite reasons to suspect that the person is spreading infection before the time for the next certificate becomes due, when an intermediate examination may be called for.
10. All such examinations must be carried out free of charge.

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ADDENDUM : *Received 14 April, 1915*

Since the above was written, eighty more cultivations have been made; from two of these the *Bacillus typhosus* was obtained in pure culture. Thus, from the total of 200 there have been six cases from which the bacillus has been isolated, apart from those subjects who

showed post-mortem signs of enteric fever. Of these two, one was a patient dying of dysentery, the other of tuberculosis of the lungs and pleurae. In addition, therefore, to the usually recognised carriers, who have definitely passed through an attack of typhoid fever, I have found 3 per cent. of persons dying from some other affection than typhoid, from whom no history was obtained of having suffered from the disease, nevertheless harbouring the bacillus in their gall-bladders at the time of death.

H. H. S.

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TABLE II.—Post-mortem findings and results of cultivation of the Bile from 120 consecutive autopsies.

No.	Initials	Sex	Age in years	ENTERIC FEVER			DIAGNOSIS		BILE CULTURE	
				Sup- pected	Found post- mortem	Widal	Clinical	Found post-mortem	Typhoid	Others
1	R. C.	F.	8	Yes	Yes	+	Enteric fever	Enteric fever	+	—
2	E. C.	M.	54	No	No	...	Fractured ribs	Also ruptured liver and spleen	—	—
3	A. G.	M.	38	No	No	...	Sarcoma, left kidney	Double cystic kidney, left 5610, right 2250 grams	—	—
4	M. H.	F.	21	No	Yes	—	Pneumonia, left	Typhoid ulcers	+	—
5	A. T.	M.	27	No	Yes	...	Pneumonia	Typhoid ulcers, very numerous, 5 in appendix	+	—
6	L. A.	F.	40	No	No	—	Enteritis	Tuberculosis, lungs, spleen, intestine	—	—
7	C. C.	M.	48	No	No	...	Arsenical poisoning (suicidal)	Irritant poisoning, 431 grams arsenious acid	+	—
8	J. F.	M.	31	No	No	...	None made	Pulmonary tuberculosis	—	—
9	O. W.	M.	?	No	Yes	...	Pneumonia	Typhoid ulcers, old pleuritic adhesions	+	<i>B. coli</i>
10	R. S.	M.	22	No	Yes	...	General peritonitis	Peritonitis from typhoid perforation	+	<i>B. coli</i>
11	A. N.	F.	25	No	No	...	Phthisis	Tuberculosis, lungs and spleen	—	Streptococci
12	C. F.	M.	41	No	No	...	None made	Colitis	—	—
13	A. F.	M.	43	No	No	...	Dysentery	Large intestine gangrenous, mitral vegetations, renal infarcts	—	Shiga type
14	J. S.	F.	28	No	No	...	Mitral regurgitation	Mitral disease	—	—
15	Z. S.	M.	51	No	No	...	Liver abscess	Confirmed	—	<i>B. coli</i>
16	A. G.	F.	19	Yes	Yes	Too early	Enteric fever	Confirmed	+	—

TABLE II.—continued.

No.	Initials	Sex	Age in years	ENTERIC FEVER			DIAGNOSIS		BILE CULTURE	
				Suspected	Found post-mortem	Widal	Clinical	Found post-mortem	Typhoid	Others
17	E. W.	...	25	No	No	...	Phthisis	Confirmed	-	-
18	S. D.	...	25	No	No	...	None made	Tuberculosis, left lung, small intestine	-	-
19	L. B.	...	25	No	No	-	Phthisis	Empyema, left; fibrino-purulent pericarditis	-	-
20	E. N.	...	22	No	No	...	Not made	Mitral disease	-	-
21	R. C.	...	?	No	No	...	Chronic nephritis	Confirmed	-	-
22	E. P.	...	21	Yes	Yes	+	Enteric fever	Confirmed	+	-
23	L. G.	...	34	Yes	Yes	+	Enteric fever	Confirmed	+	-
24	A. W.	...	53	No	No	...	Chronic nephritis	Confirmed	-	-
25	G. M.	...	7/12	No	No	...	Congenital syphilis	Confirmed	-	-
26	J. M.	...	30	No	No	-	Subtertian malaria	Confirmed	-	-
27	P. M.	...	49	No	No	...	Urethral stricture	Chronic nephritis, right lobar pneumonia	-	-
28	M. H.	...	40	No	No	...	None made	Mitral Incompetent	-	-
29	M. McC.	...	?	No	No	...	Nephritis and cardiac	Confirmed	-	-
30	E. R.	...	19	Yes	Yes	+	Enteric fever	Confirmed	+	-
31	V. W.	...	12	No	No	...	'Vomiting sickness'	Tubercular enteritis and peritonitis	-	<i>B. coli</i>
32	E. J.	...	19	Yes	No	-	None made	Phthisis	-	-
33	M. M.	...	24	Yes	Yes	+	Enteric fever	Confirmed	-	-
34	B.	...	21	No	No	...	Phthisis	Liver abscesses, gall-stone ulcerated into duodenum	+	-

TABLE II.—continued.

No.	Initials	Sex	Age in years	ENTERIC FEVER			DIAGNOSIS		BILE CULTURE	
				Suspected	Found post-mortem	Widal	Clinical	Found post-mortem	Typhoid	Others
35	K. C.	F.	20	Yes	Yes	+	Enteric fever	Confirmed	+	—
36	J. S.	M.	21	No	No	...	Periculous anaemia	Gangrenous appendicitis	—	—
37	A. B.	F.	46	No	No	...	Lipoma left groin	Richter's hernia strangulation	—	—
38	V. C.	M.	20	Yes	Yes	+	Enteric fever	Confirmed	—	<i>B. coli</i>
39	A. S.	F.	29	No	No	...	Pneumonia	Tuberculous broncho-pneumonia	—	—
40	G. T.	M.	33	No	No	...	Extravasation of urine	Confirmed	—	<i>B. coli</i>
41	E. G.	F.	12	Yes	No	+	Enteric fever	Broncho-pneumonia ? typhoid	—	—
42	F. R.	M.	40	No	No	...	Epithelioma of penis	Confirmed	—	—
43	M. B.	F.	4	Yes	Yes	Indefinite	Enteric fever	Confirmed	+	—
44	W. S.	M.	16	Yes	? old pigmented Peyer's patches	+	Enteric fever	Tuberculosis, pleura, pericardium and bronchial glands	—	—
45	R. J.	M.	23	Yes	Yes	+	Enteric fever	Confirmed	+	—
46	A. J.	F.	5	No	No	—	Meningitis	Abscess of lung pyo-pneumothorax	—	Streptococci
47	J. J.	M.	40	No	No	...	Phthisis	Confirmed	—	—
48	A. F.	F.	20	Yes	Yes	+	Enteric fever	Confirmed	—	<i>B. parat. A.</i>
49	A. McM.	M.	7	No	No	...	Tuberculosis of lungs	Confirmed	—	—
50	V. H.	F.	15	Yes	Yes	+	Enteric fever	Confirmed	+	—
51	C. G.	M.	46	No	No	...	Stricture and chronic nephritis	Confirmed	+	<i>B. coli</i> and streptococcus



TABLE II.—continued.

No.	Initials	Sex	Age in years	ENTERIC FEVER			DIAGNOSIS		BILE CULTURE	
				Sus-pected	Found post-mortem	Widal	Clinical	Found post-mortem	Typhoid	Others
52	A. T.	M.	14	No	No	...	Dysentery	Confirmed (amoebic)	-	Streptococci
53	S. H.	F.	42	No	No	...	Carcinoma, uterus	Confirmed	-	-
54	F. P.	M.	60	No	No	...	Cystitis	With enlarged prostate	-	-
55	A. F.	F.	24	Yes	No	-	Enteritis	Phthisis and ankylostomiasis	-	-
56	J. M.	F.	17	Yes	Yes	-	Not made	Typhoid ulcers, early stage	+	-
57	M. B.	F.	16	No	No	-	Double pneumonia	Right pyo-salpinx, septicaemia embolic pulmonary abscesses	-	-
58	N. N.	F.	41	No	No	...	Sarcoma, upper jaw	Confirmed	-	-
59	J. S.	M.	?	No	No	...	Uraemia	Confirmed, contracted granular kidney	-	-
60	J. W.	M.	20	Yes	Yes	+	Enteric fever	Confirmed	+	-
61	A. D.	F.	15	No	No	...	Not made	Tuberculosis, lungs and intestine	-	-
62	L. G.	M.	2½	No	No	...	Enteritis	Confirmed	-	<i>B. lactis aerogenes</i>
63	W. S.	F.	30	No	No	...	Intestinal obstruction	Band from old appendicitis	-	-
64	J. B.	M.	10	Yes	Yes	+	Enteric fever	Confirmed	-	<i>B. coli</i> only
65	J. D.	M.	64	No	No	...	Cellulitis of hand	Confirmed, also mitral disease	-	-
66	T. C.	M.	24	No	No	...	Acute Pneumonia	Confirmed	-	<i>B. coli</i>
67	R. T.	F.	11	Yes	Yes	+	Enteric fever	Confirmed	+	-
68	Rh. T.	F.	19	Yes	Yes	...	Enteric fever	Confirmed	-	-
69	E. T.	F.	21	Yes	No	-	Not made	General tuberculosis, lungs, peritoneum, pleura, liver, intestines	-	Streptococci

TABLE II.—continued.

No.	Initials	Sex	Age in years	ENTERIC FEVER			DIAGNOSIS		BILE CULTURE	
				Suspected	Found post-mortem	Widal	Clinical	Found post-mortem	Typhoid	Others
70	E. B.	F.	22	No	No	...	Puerperal septicaemia	Confirmed	-	Streptococci and <i>B. coli</i>
71	A. McN.	M.	22	Yes	Yes	+	Enteric fever	Confirmed	+	-
72	S. Y.	M.	40	No	No	...	Arsenic poisoning (suicide)	Confirmed	-	-
73	S. D.	M.	19	No	No	-	Lobar pneumonia	Confirmed	+	-
74	A. C.	F.	30	Yes	No	-	Not made	Colitis ? dysenteric	-	<i>B. coli</i>
75	Not known	M.	?	No	No	...	Not made	Cerebral haemorrhage	-	-
76	J. B.	M.	40	No	No	...	Pneumonia	Confirmed	-	-
77	J. S.	F.	19	No	No	...	Mitral disease and phthisis	Confirmed	-	-
78	L. S.	F.	50	No	No	-	Not made	Malignant endocarditis, embolic pyaemia	-	-
79	J. R.	M.	30	No	No	...	Not made	Tuberculosis, lungs and serous membranes	-	-
80	M. W.	F.	19	Yes	No	-	Not made	Tuberculosis, lobar pneumonia	-	-
81	F. J.	F.	23	No	No	...	Pyæmia	Confirmed	-	Streptococci
82	J. J.	F.	17	Yes	No	-	Not made	Large pale kidney ? uraemia	-	-
83	W. A.	M.	48	No	No	...	Not made	Phthisis	-	-
84	W. S.	M.	19	Yes	No	?	Enteric fever	Phthisis	-	<i>B. coli</i>
85	J. P.	M.	? 25	No	No	-	Not made	Glioma, right hemisphere	-	-
86	A. R.	F.	38	Yes	No	-	? Enteric fever	Cirrhosis of liver, chronic intestinal nephritis	-	<i>B. coli</i>
87	W. B.	M.	72	No	No	-	Gangrene leg	Confirmed	-	-

TABLE II.—continued.

No.	Initials	Sex	Age in years	ENTERIC FEVER		Widal	Clinical	DIAGNOSIS	BILK CULTURE	
				Suspected	Found post-mortem				Typhoid	Others
88	J. B. L.	M.	35	No	Yes	...	Cerebral haemorrhage	Enteric fever	+	—
89	M. L.	F.	38	No	No	...	Fibroid uterus	Also phthisis, and old pleural effusion, left	—	—
90	T. W.	M.	52	No	No	...	Cerebral haemorrhage	Cerebellar tumour	—	—
91	A. McG.	F.	62	No	No	...	Malignant disease	Carcinoma of uterus and peritoneum	—	—
92	P. L.	F.	42	No	No	...	Intestinal obstruction	Volvulus	—	<i>B. coli</i>
93	C. E.	F.	? 22	No	No	...	Peritonitis	Confirmed, perforation of malignant growth of jejunum	—	Morgan
94	D. D.	M.	20	Yes	No	—	Enteric fever	Dysentery	—	—
95	T. M.	F.	26	No	No	...	Not made	Fibroids, and early pregnant uterus (death from haemorrhage)	—	<i>B. coli</i>
96	C. V.	M.	27	No	No	...	General peritonitis	Confirmed, perforated duodenal ulcer	—	—
97	H. W.	F.	37	No	No	...	Pleural effusion	Malignant growth right lung and pleura	—	—
98	S. H.	M.	2	No	Yes	...	Not made	Enteric fever	+	—
99	A. G.	F.	19	No	Yes	...	Pneumonia	Confirmed, but also enteric fever	+	—
100	J. B.	F.	19	No	No	...	Cerebral malaria	Tuberculosis, lungs and bronchial glands	—	—
101	E. L.	F.	19	No	No	...	Drowning	Confirmed	—	—
102	E. D.	M.	16	Yes	No	—	? Dysentery	Ulcerative colitis	—	—
103	M. B.	F.	12	No	No	...	Not made (moribund)	Dysentery	—	—

TABLE II.—continued.

No.	Initials	Sex	Age in years	ENTERIC FEVER			DIAGNOSIS		BILE CULTURE	
				Suspected	Found post-mortem	Widal	Clinical	Found post-mortem	Typhoid	Others
104	J. W.	M.	25	No	No	...	Phthisis	Confirmed	-	<i>B. coli</i>
105	C. R.	M.	20	Yes	Yes	+	Enteric fever	Confirmed	+	-
106	Ch. R.	M.	22	Yes	No	-	Enteric fever	Empyema	-	-
107	G. S.	F.	17	Yes	Yes	+	Enteric fever	Confirmed	+	-
108	W. B.	M.	? 60	No	No	...	Fractured base	Intravent. haemorrhage	-	-
109	H. W.	F.	21	No	No	...	Not made	Ulcerative colitis ? dysentery	-	-
110	E. H.	M.	36	No	No	...	Phthisis and pericarditis	Lobar pneumonia	-	-
111	T. S.	M.	31	No	No	...	Asthma	Aortic aneurism	-	-
112	C. B.	M.	39	No	No	...	Morbis cordis	Aortic and mitral	-	-
113	E. R.	F.	21	No	No	...	Chronic nephritis	Confirmed	+	-
114	W. M.	M.	38	No	No	...	Amoebic dysentery	Confirmed	-	<i>B. lactis aërog.</i>
115	J. M.	M.	75	No	No	...	Epilepsy and phthisis	Chronic intestinal nephritis, no cp.	-	<i>B. coli</i>
116	V. W.	F.	28	No	No	...	Mitral disease	Confirmed	-	-
117	C. F.	M.	13	No	No	...	Pneumonia following fall into sea off pier	Empyema and pulmonary abscess	-	<i>B. lactis aërog.</i>
118	S. H.	F.	25	Yes	Yes	...	Enteric fever	Confirmed	+	-
119	F. W.	M.	13	No	No	...	Paraplegia	Myelitis (traumatic)	-	-
120	R. S.	F.	26	No	No	...	Mitral regurgitation	Confirmed	-	-

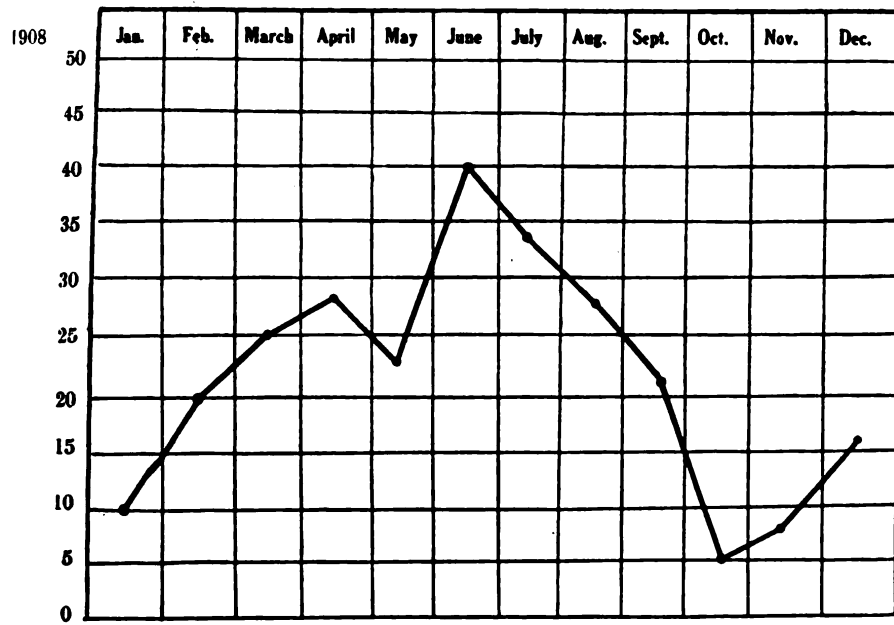


CHART II.—Notifications of Enteric Fever cases in Kingston during 1908.

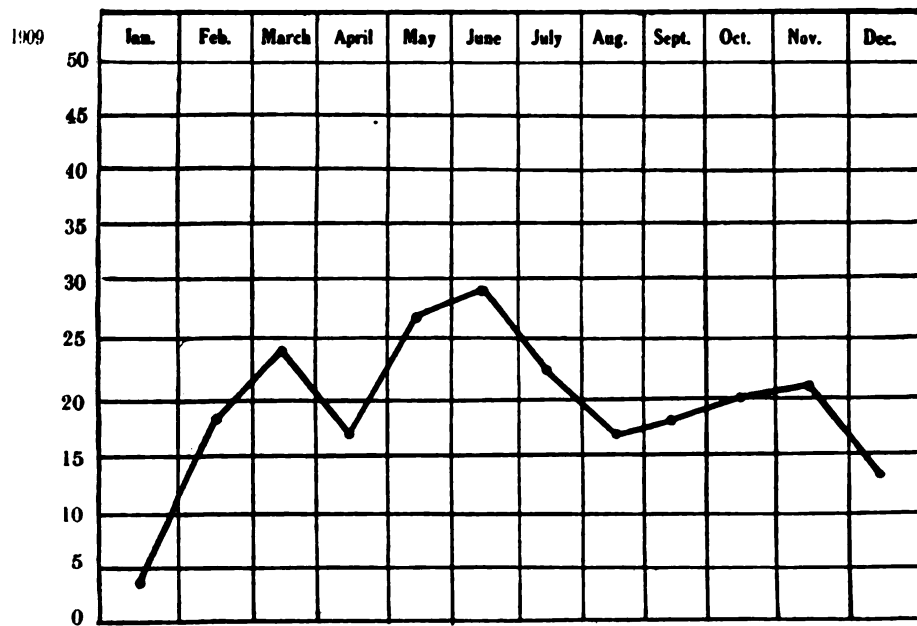


CHART III.—Notifications of Enteric Fever cases during 1909.

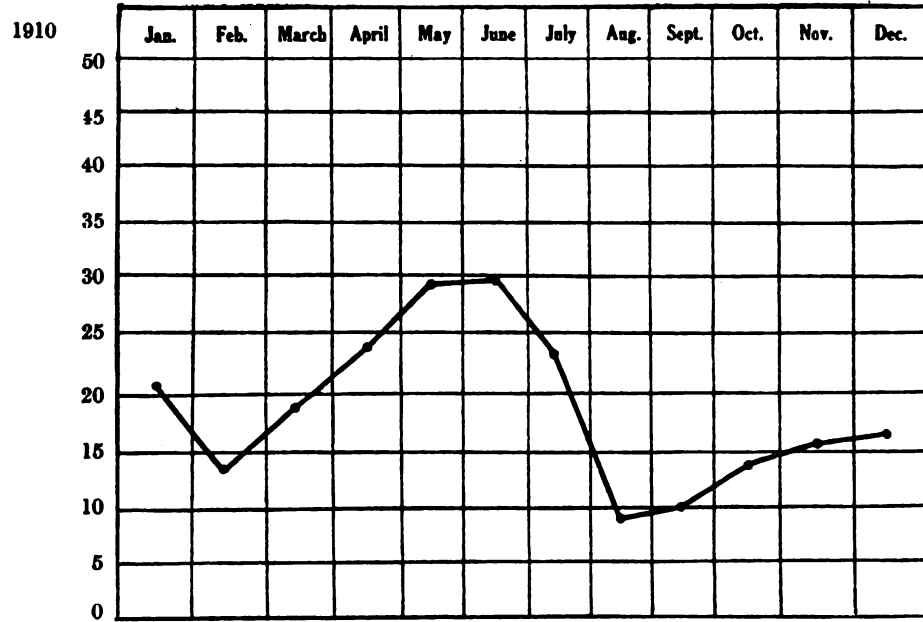


CHART IV.—Notifications of Enteric Fever cases during 1910.

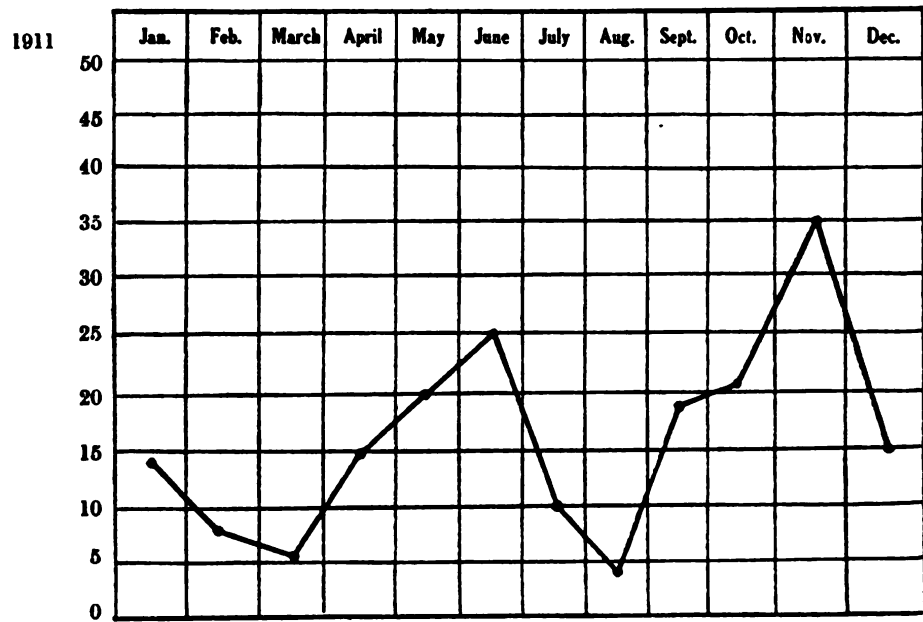


CHART V.—Notifications of Enteric Fever cases during 1911.

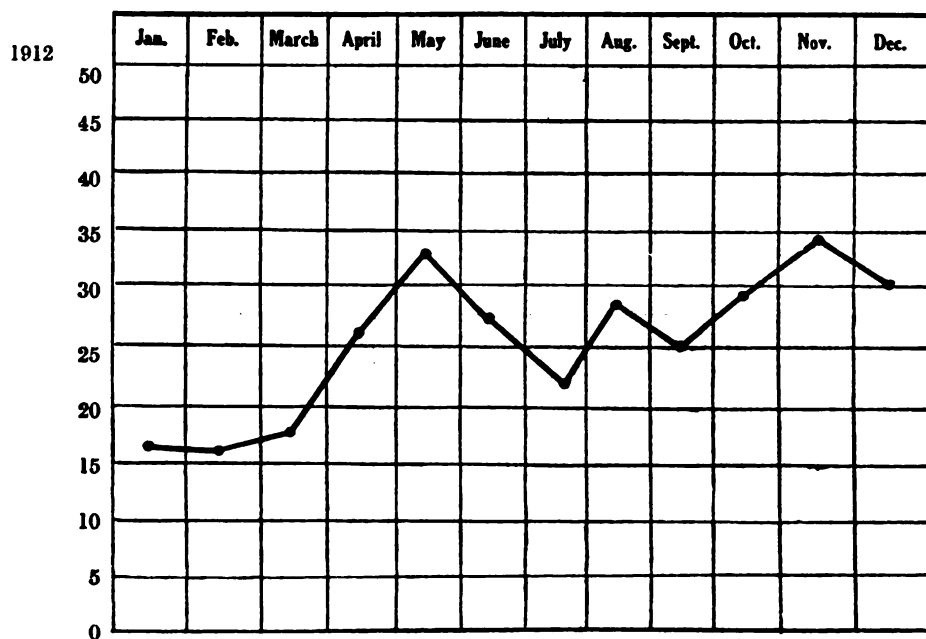


CHART VI.—Notifications of Enteric Fever cases during 1912.

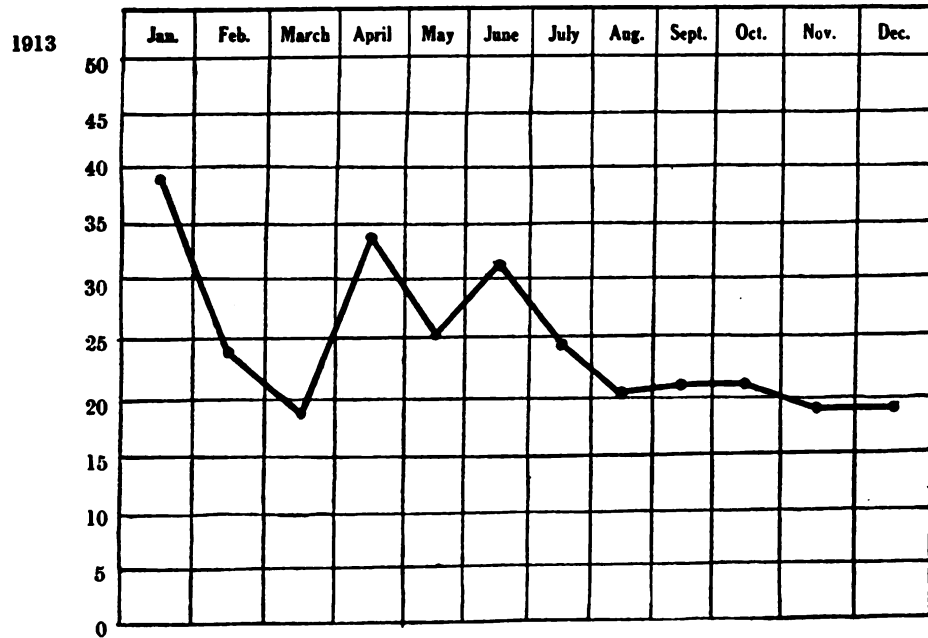


CHART VII.—Notifications of Enteric Fever cases during 1913.

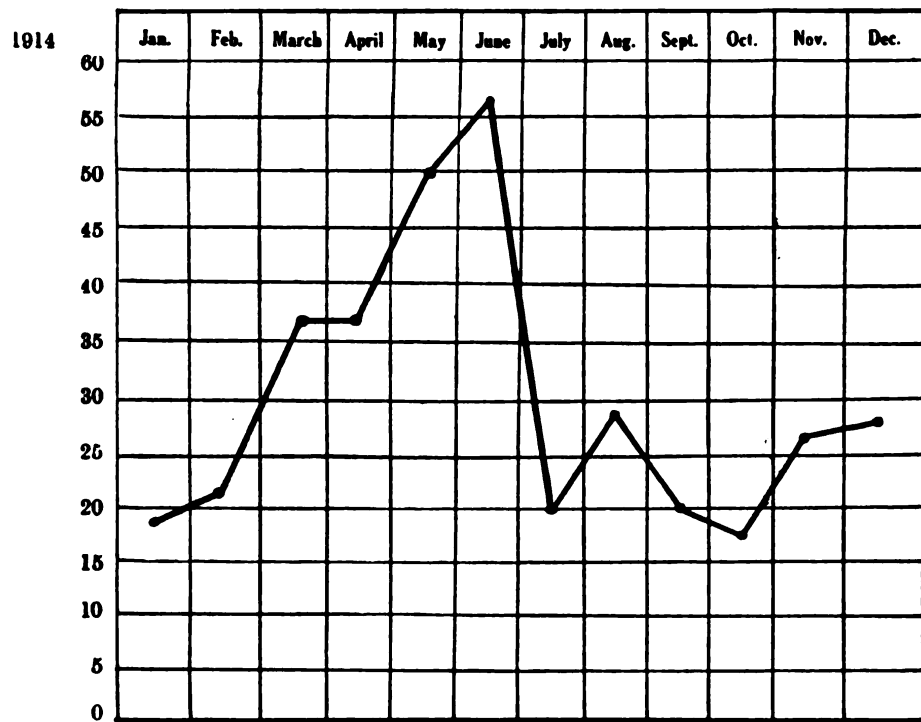


CHART VIII.—Notifications of Enteric Fever cases during 1914.



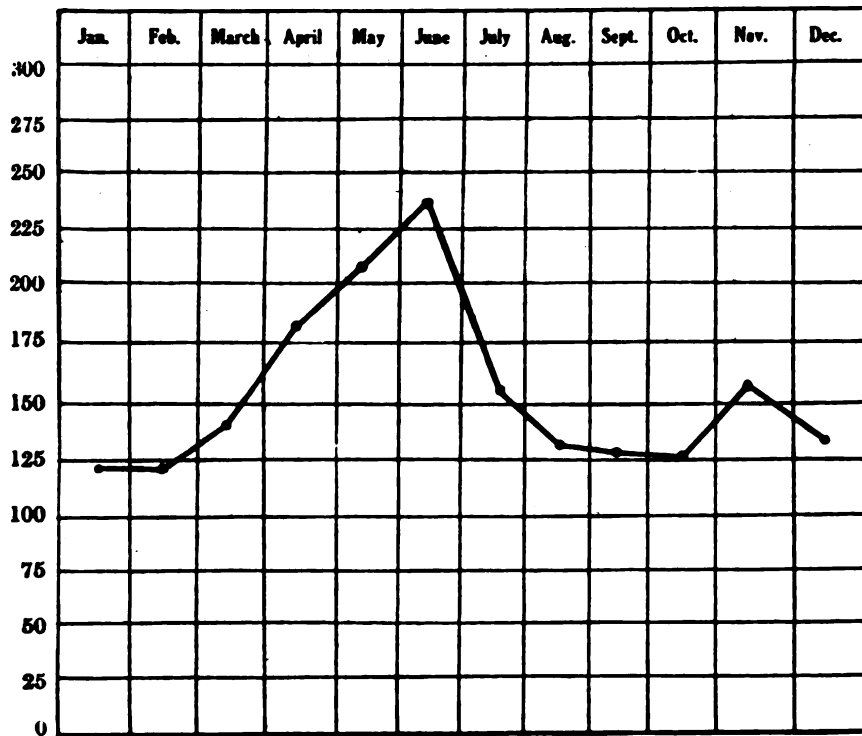


CHART IX.—Total numbers of notifications of Enteric Fever cases in Kingston, month by month, for the years 1908-1914.

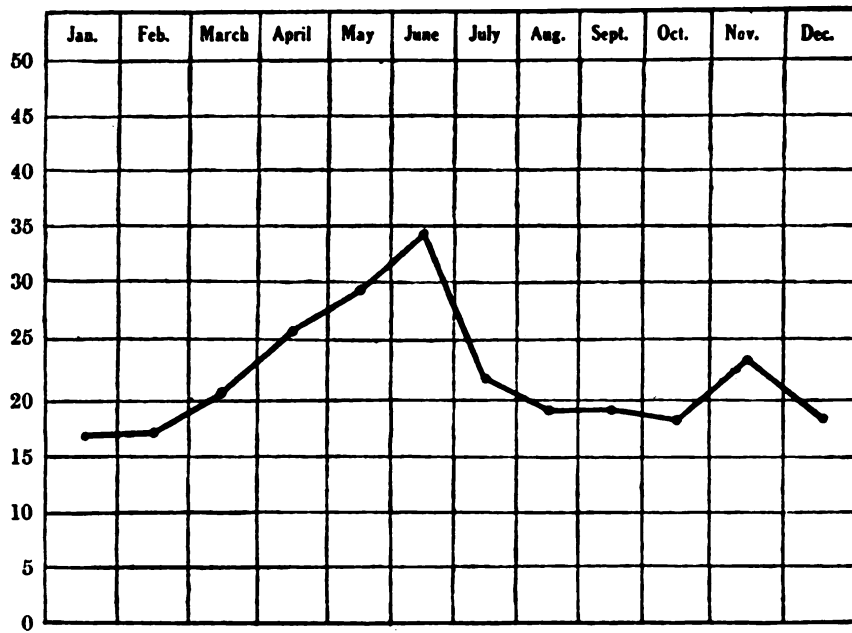


CHART X.—Average of notifications of Enteric Fever cases in Kingston, month by month, for the years 1908-1914.

# ON THE OCCURRENCE AND PRE- VALENCE OF DISEASES IN BRITISH NEW GUINEA

BY

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FROM THE AUSTRALIAN INSTITUTE OF TROPICAL MEDICINE

*(Received for publication 4 February, 1915)*

PLATES XIX—XXVI AND MAP

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## INTRODUCTION

In the following pages are embodied the scientific results of two journeys to the coastal belt of British New Guinea, which were undertaken for the purpose of mapping out the incidence and geographical distribution of tropical diseases among the natives.

The first journey took place during the months of July and August, 1912, and included the coastal belt east of Port Moresby and north of Samarai as far as the Mambare River.

During the second journey, lasting from the end of June to the beginning of October, 1913, the coastal regions west of Port Moresby as far as Daru were visited. To the Purari River the journey was accomplished on foot, and continued from there in native canoes.

The coast of New Guinea east of Port Moresby is fairly densely populated, the natives living in numerous villages, ranging in size from three or four houses to large settlements with approximately 3,000 inhabitants, although very large villages are rare.

The majority of the natives on the south-east coast live by agriculture and fishing. The villages consist of a number of houses sufficiently large to provide a comfortable shelter for one family. Some of the villages are built on high piles in the sea, the houses being connected by bridges, but most of the traffic between the houses is carried on by native canoes.

In a great number of the villages situated further inland the houses are built on both sides of a main street, since the Government advises, for obvious reasons, this scheme of laying out the villages. The majority are built on sandy soil and surrounded by more or less dense scrub or jungle.

The coastal districts west of Port Moresby, as far as Orokolobay, are of the same nature as those on the south-east coast. The villages are densely dotted all along the coast, and in many instances a few miles only intervene between neighbouring villages.

After passing Orokolobay the nature of the country undergoes a complete change. The coast between the Purari and the Fly Rivers is broken up into a continuous delta. There are a number of large rivers connected by numerous cross channels and cross streams, and the land is entirely of a muddy and swampy nature. The villages are built on mud flats, and the traveller may journey for days and weeks without stepping on firm soil.

The villages there consist of houses of varying size; besides small huts accommodating one family only, there are very large houses, inhabited by a number of families, and large club houses where the unmarried boys dwell.

Throughout the west the native houses are built on high piles, and the different sections of each village are connected by bridges, consisting of a light scaffolding with loose saplings laid across, which are renewed at rare intervals. Still further west, on the Fly River,

each village consists of a small number of very large houses which give shelter to a hundred or more people, and are partitioned off into cubicles each occupied by a single family.

Generally speaking, the natives of the south-east and west coasts as far as Orokelo are highly civilized, and have been in constant contact and communication with Europeans for nearly a quarter of a century. West of Orokelo the natives become less and less civilized. In some districts, especially in the neighbourhood of the Kikori and Turama Rivers, numbers of villages are not under Government control, and many of them have not even been visited by Europeans. The villages situated in the Fly Estuary, and the inhabitants of some of the islands, as Kiwai Island, are as highly civilised as the natives in the neighbourhood of the capital of British New Guinea, Port Moresby.

The medical survey was carried out with all possible thoroughness and care, and it may be assumed that cases of almost all diseases prevalent in New Guinea have been encountered in one village or another.

During the journey a number of blood specimens were obtained in order to map out the distribution of filariasis and malaria.

The less civilised the districts, the fewer natives were found willing to supply a drop of blood for a film. Unfortunately in several districts, especially around the Kikori River, it was impossible even to obtain photographs of natives suffering from the various complaints. As opportunity offered, sores were scraped with a sharp spoon, and the granulation tissue so obtained preserved in 70 per cent. alcohol for future microscopical examination.

Speaking generally, a number of diseases such as various types of ulcers, malaria, filariasis and elephantiasis, juxta-articular nodules, gangosa, yaws, and several skin diseases, mainly *tinea imbricata*, were encountered in most of the villages visited. It must be borne in mind that for a considerable time frequent intercourse and trade took place amongst the natives on the south coast between Orokelo and Samarai. Every year the natives of Port Moresby and several adjoining villages, who are the potters of New Guinea, travel west in the lakatois (large sailing canoes), exchanging their pots for sago which grows abundantly in the western parts. The journeys must have offered ample opportunity for the spread of contagious

and infectious diseases. In more recent times the opening up of the country, and the drawing of the supply of native labour from districts the inhabitants of which had hitherto not been wont to travel, has given rise to new channels for the propagation of disease. It is, however, noteworthy that several diseases, such as gangosa, juxta-articular nodules, yaws and tinea imbricata, and different types of sores, were seen in natives who were unable to travel any considerable distance without endangering life and limb on account of hostile neighbours.

### **MALARIA**

With the exception of a few districts, malaria occurs with varying severity throughout British New Guinea, although its frequency is dependent on seasons and local conditions. New Guinea, situated within the monsoonal belt, has two distinct seasons—the north-west beginning at the end of November and lasting until March or April, when land breezes prevail, and the south-east season when the prevailing wind comes from the sea.

In the coastal villages malarial fever is more prevalent during the north-west season when mosquitoes are in evidence. During the south-west time, on the other hand, malaria is far less common, since the sea breeze prevents the mosquitoes from swarming into the villages.

In the inland villages in sheltered positions, on the whole, no marked seasonal variation is observed. Coastal villages in exposed positions are comparatively free from fever, whereas coastal villages sheltered from the breeze show a large percentage of children with enlarged and palpable spleens. In the villages west of Orokolo, built on mud flats, which were visited during the south-west monsoon, no cases of malarial fever amongst the natives were encountered, although fever is said to be prevalent during the north-west season.

The natives contract malaria as a rule in early childhood, and acquire, in the course of years, an active immunity against the type of malarial infection prevalent in their place of residence. The incidence of malaria in natives decreases in direct proportion to their age.

The number of children with enlarged spleen gives a definite

indication of the amount of malaria in a given locality. The spleen census was taken by determining the number of children with enlarged spleens in the different villages, and blood films were made of those children who showed considerable enlargement of the spleen to ascertain the type of malaria prevalent.

On several occasions the spleen index of babies in arms was taken separately from that of children of ages ranging between 5 and 11 years, and the former was considerably higher than the latter, in one instance 95.1 in comparison with 58.6.

The accompanying map shows that simple and malignant tertian types of malaria are fairly equally distributed and more common than the quartan type; the latter is more prevalent in the furthest north-east corner, on the mouth of the Mambare River, and is not infrequently met with in the Mekeo district.

Out of 245 blood films taken from a small number of native children, 132 contained malarial parasites, sometimes in considerable numbers; at other times only a few ring forms were seen in a whole film.

Out of 245 blood films examined, 48 contained the parasite of malignant tertian, 34 of simple tertian, and 27 of quartan malaria. In 23 the type of the parasite could not be determined with certainty.

The white population of British New Guinea suffers, with hardly any exception, from occasional attacks of malarial fever, and not one white settler was met who had been entirely free from attacks of fever during his stay in the country.

Of Anophelinae, *Nyssorhynchus annulipes* and *Cellia punctulata* were collected, the former being the more common mosquito, occurring in the proportion of about 10 to 1 of the latter.

The occurrence of *Nyssorhynchus annulipes* in numbers throughout the districts where malarial fever is most prevalent, and the fact that the development of *Plasmodium falciparum* has been traced by Kinoshita (1906) in this mosquito, make it extremely likely that *Nyssorhynchus annulipes* acts as intermediary host of the malarial parasite in New Guinea.

Of other Anophelinae, *Nyssorhynchus bancrofti* has been collected, but this species does not transmit the parasite.

## FILARIASIS

Thorpe in 1896 drew attention to the fact that the microfilaria in the blood of the natives of the Tonga Islands does not show the same periodicity as observed elsewhere. Lynch (1905) confirmed this statement, finding that in 105 out of 156 cases of filariasis amongst the Fijians, microfilariae were present in the peripheral blood both by day and night.

Similar observations in the cases of filariasis among the natives of the Philippine Islands led Ashburn and Craig (1907) to the creation of a new species, *Filaria philippensis*. The larvae, although resembling closely *Microfilaria nocturna*, do not possess any periodicity, and occur in approximately the same numbers in the peripheral blood during all hours of the night and day. The movements of this microfilaria are described as progressive and lashing, the sheath is very tightly fitting, so that the microfilaria cannot slip backwards and forwards within it, as in the case of *Microfilaria nocturna*. At the posterior end the tail diminishes progressively and uniformly to a fine thread-like point. Bahr (1912), Fülleborn (1913), and many other observers, have failed to discriminate between the larvae of *Filaria philippensis* and that of *Filaria bancrofti*.

Fülleborn (1911), utilising material collected in the Bismark Archipelago and German New Guinea, pointed out that 10 to 60 per cent. of the natives showed large sheathed microfilariae in their blood, which were of the same anatomical structure as *Microfilaria bancrofti* but did not possess the typical nocturnal periodicity. Moreover, the adult worm of the Fijian microfilaria, according to Leiper, cannot be differentiated from *Filaria bancrofti*.

Fülleborn (1912) concluded, therefore, that in the present state of our knowledge two biological varieties of *Filaria bancrofti* can be differentiated; the larvae of one variety have a pronounced nocturnal periodicity, whereas the larvae of the other, mostly occurring in the Pacific, are present in the peripheral blood at all hours of the day and night.

A troop of twenty-three Samoans, who were visiting Europe, were examined by Fülleborn (1912). He found that nearly half of them harboured microfilariae in their peripheral blood, morphologically



identical with *Microfilaria nocturna*, but without any marked periodicity.

Whilst travelling through the coastal districts of British New Guinea, thin smears were made from the day blood of a number of men, women and children to be examined for the presence of microfilariae. It was found impossible to use thick films for this work, since previous experience had shown that they deteriorate much quicker than thin blood smears, and after exposure for some time to the moist tropical heat are difficult to dehaemoglobinize. On account of the short stay in the different villages it was possible to take blood films in the day-time only, as the blood examination of the same natives on two occasions would have meant too great a delay. A more thorough and extensive filarial survey will be undertaken at a future date.

Although the number of blood examinations was comparatively small, the results indicate that the natives living in the coastal belt of eastern and north-eastern New Guinea harbour filarial larvae in their blood to a greater extent than in the western parts. Out of 166 blood slides taken at random on the first journey, twenty-four contained microfilariae, giving an infection index of slightly over 17 per cent., whereas out of 166 films taken during the second journey, microfilariae were discovered in eight slides, being about 5 per cent. A more extensive survey, taking samples both in the day and night time, would almost certainly reveal a much larger percentage of infection.

On two occasions only the blood of some of the carriers was examined, and two and three respectively, out of about fifteen, proved to be infected with filarial larvae, which showed the typical nocturnal periodicity.

Morphologically the microfilaria found in the blood of New Guinea natives does not differ essentially from typical *Microfilaria nocturna*. Table 1 gives the measurements of a number of microfilariae, stained by Giemsa's method, and for comparison are appended Fülleborn's average measurements of typical *Microfilaria nocturna* and of microfilariae found in the blood of Samoans.

The experience gained in New Guinea coincides with Bahr's observations in Fiji, and Fülleborn's in the South Pacific, that there exists in British New Guinea a microfilaria morphologically identical

TABLE I.—Measurements of *Microfilariae* found in the blood of New Guinea natives; films taken in the daytime.

	Nerve spot from anterior end	Excretory pore from anterior end	Inner body from anterior end	Length of inner body	Gi cell from anterior end	Anal pore from anterior end	Total length
	%	%	%	μ	%	%	μ
1	18.5	28.1	46.8	50	...	81.4	405
2	19.1	28.5	52.3	50	73.8	88.0	420
3	17.3	27.9	48.6	38	...	84.8	358
4	16.5	26.6	48.9	60	68.9	85.3	450
5	20.1	...	57.4	35	74.1	...	340
6	19.3	28.6	45.7	70	73.4	...	350
7	19.6	29.4	40.0	60	...	86.8	305
8	16.4	26.2	47.8	55	73.8	87.3	366
9	18.2	27.3	50.0	45	71.5	82.9	440
10	21.0	29.5	51.1	52	74.5	88.0	352
11	19.6	31.6	60.7	30	74.4	88.5	395
12	18.5	30.3	48.4	60	75.7	...	330
13	...	28.0	49.6	56	...	87.0	310
14	18.4	29.0	50.4	54	71.4	...	245
Average	18.7	28.5	49.9	51	73.1	86.0	362

Fülleborn's average measurements of typical *Microfilaria nocturna*—

18.3	28.6	...	...	70.6	83.5	232.5
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Average of non-periodical Samoan *Microfilaria*—

19.3	28.5	...	...	70.2	82.9	272.5
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with *Microfilaria nocturna*, but not possessing the typical periodicity, in addition to microfilariae which are only present in the peripheral blood at night time.

It is interesting to note that only one specimen of *Stegomyia pseudoscutellaris* was collected in Samarai on the south-east coast of New Guinea, which species was not encountered anywhere else.

The patchy distribution of filariasis is quite as well marked in New Guinea as in other parts of the tropics; for example, in one village nearly 3 to 5 per cent. of the male population suffered from a soft swelling of the groin glands, so typical in filariasis, whereas in the neighbouring villages cases were only rarely noticed.

Cases of elephantiasis were seen in varying numbers throughout the districts visited. Of nearly every case of elephantiasis blood films were taken, but in no instance were microfilariae found in the blood. The arms and legs were most frequently affected, the arms perhaps more often than the legs. The genitalia were not so frequently implicated.

Plate XIX, fig. 1, shows a typical and far advanced case of elephantiasis of the scrotum, affecting at the same time one leg; and fig. 2 shows a typical case of elephantiasis of the labium minor of the vulva.

### LEPROSY

The distribution of leprosy in New Guinea is of some interest. This disease has already been referred to by W. MacGregor in the yearly report for New Guinea, 1888-1889, where it is stated that 'Leprosy is not an uncommon disease amongst the natives, but does not present its worst form and has not been communicated to any white person.'

On the whole, in the districts visited, leprosy was found endemic in only three, namely, the Trobriand Islands—a number of islands off the north-east coast—the Mekeo district on the St. Joseph River in the Central Division of British New Guinea, and in one district of the Gulf division.

Only very few cases were seen on the south coast east of Port Moresby, and not one definite case west of the Vailala River. Altogether eighteen cases were diagnosed, four cases in the Trobriand Islands, seven in the Mekeo district, and eleven in the

villages on the south coast between Isiu and Orokolo, west of Port Moresby.

The three different types, namely, nodular, nerve, and mixed leprosy, were found.

Plate XIX, fig. 3, shows one of the most advanced cases of leprosy seen.

It is an interesting fact that the natives of the Trobriand Islands know leprosy well, having a special native name for the disease, namely, Kai-gwai-guia. When the natives were asked to bring forward these cases, all produced were typical lepers.

Considering the number of natives seen, and the large area traversed, it becomes apparent that leprosy is present in New Guinea, although by no means prevalent. A great number of ulcers of various descriptions resembled leprosy at the first glance, but careful examination excluded the diagnosis.

#### JUXTA-ARTICULAR NODULES

The occurrence of juxta-articular nodules was first described by MacGregor in 1901 in New Guinea. Later, in 1904, Steiner described the occurrence of tumours amongst Javanese, appearing as hard, round irregular nodules below the skin, and situated always in the neighbourhood of the joints. The skin above the tumours was moveable, and the nodules were not connected with the underlying fascia. On histological examination these nodules consisted of concentric layers of hard fibrous tissue, which was degenerated in the centre into coarse, irregular, structureless masses. Various staining methods did not reveal the presence of any bacteria.

Two years later, Jeanselme (1906) described the occurrence of similar tumours amongst the natives of Siam. Microscopic examination showed that they were formed of three layers—a central one consisting of degenerated tissue, an outer zone of inflammatory reaction, and an intermediate or transitional zone.

Gros (1907) observed the occurrence of similar tumours amongst the Algerian natives in small numbers only; ten cases were seen out of approximately 12,000 patients. Histologically they were of similar structure to those examined by Jeanselme.

The occurrence of the same disease was reported later by Neveux

(1907) amongst the natives of Senegambia, and Fontoynont and Carougeau (1908) made an extensive study of this complaint in Madagascar. In their experience, juxta-articular nodules were found in persons of both sexes and of all ages, though the male sex was more frequently attacked by the disease. Special attention was drawn to the symmetrical occurrence of these nodules. They pointed out that the seat of predilection of the tumours was the outer surface of the extremities, especially the extensor surfaces, also, that they occurred in groups in the skin in the neighbourhood of joints, and especially where bone lies immediately below the skin, as near the elbow and large trochanter.

The development of the juxta-articular nodules is very slow, extending over years, until finally they become fibrous or fibro-cartilaginous. Fontoynont and Carougeau found in early nodules, amongst the soft caseous central mass, very small white granules, consisting of mycelium and filaments of a fungus, which they described and termed *Discomyces carougeaui*.

Inoculation of this fungus into monkeys, rabbits, and guinea-pigs, gave no results.

Lebouef (1911) observed four cases of this disease in New Caledonia. According to Joyeux (1913) similar tumours were frequently seen in natives of French Guiana, though the microscopical picture differed in some essential points from that given by Fontoynont and Carougeau. These tumours consisted of a ground substance of fibrous tissue contained within inflammatory foci, mostly around the blood-vessels. The absence of bacteria in the sections caused Joyeux to consider juxta-articular nodules as a clinical syndrome resulting from widely different causes.

Juxta-articular nodules are of frequent occurrence throughout the coastal districts of British New Guinea. In a great number of villages many cases were seen, only a few in others. The clinical manifestation did not differ from that described by previous observers. Fibrous tumours of varying size, from a pigeon's to a hen's egg, occurred in different parts of the body, mainly in the neighbourhood of joints and most frequently near the ankle joints (fig. 4) above the large trochanter and over the olecranon (Plate XX, fig. 5).

The subcutaneous tumours were round or oval, the skin above

freely moveable, and they were not connected with the underlying muscle.

They were of hard consistence, having as a rule a smooth, rarely an irregular, surface, and occurred in the majority of cases bi-symmetrically. In no cases were fistulous openings observed.

The majority of the affected natives were middle-aged men, only rarely women. One young girl, about 16 years of age, showed well-developed nodules over both elbow joints.

In some villages situated in the eastern end of British New Guinea as many as 7 to 10 per cent. of the whole population were affected. In other villages closely adjoining even the most painstaking search did not reveal the existence of a single case.

These tumours never caused the natives any pain or inconvenience, and it was only after a great deal of persuasion that permission was given to extirpate one of these tumours for histological examination. Further material was received through the kindness of Dr. Giblin in Samarai.\*

Macroscopically, in cross sections, the nodule does not differ in any respect from a subcutaneous fibroma; it consists of strands of connective tissue, concentrically arranged, the skin above being not connected with the tumour, which is apparently not encapsulated. Here and there are small irregular areas of yellowish appearance, showing softening.

On microscopical examination of three nodules, the three zones described by the previous observers could not be distinguished.

One of the nodules, which was extirpated with the overlapping skin, showed that the nodule itself was well defined from the surrounding subcutaneous tissue, which was normal except for the presence of a certain amount of small-celled infiltration and hyperaemia.

The nodule itself consisted of dense concentric layers of connective tissue cells, possessing large irregular nuclei and cytoplasm with fibrillar-like processes, which anastomosed and formed an open meshwork. Here and there, irregularly distributed throughout the nodule, were small areas where the cell nuclei had disappeared, the tissue still showing the original texture. Numbers

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\* Dr. Giblin has informed me since that he had treated one or two Europeans suffering from juxta-articular nodules.

of leucocytes were scattered throughout, but were most numerous in the degenerated parts. On the whole, the tissue of the nodules was well vascularised, and there was small-celled infiltration around the blood vessels.

The bacteriological examination of the sections did not show any microorganisms, and none of the nodules showed the '*Discomyces carougeai*.' It is probable that the fungus may only be present in the early stages, and that the specimens obtained were of cases of long duration.

### CURIOUS FEVERS

The short stay in the different villages did not afford much opportunity for studying the different types of fevers prevalent.

Four cases of a curious and apparently specific fever were observed in villages on the south-east coast. All four cases showed the same clinical symptoms, which were sufficiently pronounced to suggest a specific fever.

According to an account given by an employer of native labour, the disease sets in gradually, with an irregular fever. The first symptom noticeable is an icteric discoloration of the mucous membranes and of the sclerae, accompanied by pain in the splenic region. The patients suffer from more or less frequent attacks of continuous high fever, which may last for a few days or even a week, the temperature rising to 105° F. They emaciate considerably. The disease runs a chronic course—one of the patients had been ill for nearly two and a half years—improvement and relapses following each other at irregular intervals. The patients die finally with symptoms of great emaciation and pronounced icterus.

The examination of the four cases showed a slight enlargement of the spleen and a very marked enlargement of the liver, which in one case extended to five finger breadths below the ribs; the sclerae and the mucous membranes were of a dark yellowish colour. The blood was decidedly anaemic.

The disease is well known to the natives, and is called 'Gobora' in the Mailu district, and 'Tebi' further east.

Blood films were taken of each of the cases. Two of them showed a slight malaria infection, whilst in the others no malaria parasites could be detected. No other parasites were seen in any of the blood films.

**YAWS**

Throughout the coastal districts of British New Guinea yaws is widely distributed, and barely a single village was found entirely free from the disease. In some districts yaws is more prevalent than in others.

In 1897 Sir William MacGregor drew attention to the fact that 'Yaws is universally endemic, but it is greatly milder than it is in the Pacific, and is in fact not much taken note of. There does not even appear to be a native name for it in any dialect known. No European appears to have contracted this disease in the Possession.'

The majority of the sufferers were children, and all the typical lesions of primary and secondary yaws were seen at one time or another. It was noteworthy that in different districts one or the other of the clinical types of yaws prevailed. In the village of Orokolo, for example, many of the children examined suffered from the typical granulomatous eruption around the anus, whereas in other districts hardly one case with this manifestation was seen amongst fifty children suffering from yaws.

In the western parts sporadic cases of a generalized eruption of secondary yaws were observed. The sufferers were mostly children, and clearly showed that secondary yaws might lead to considerable destruction of the skin. In one case, a young girl (figs. 6 and 7), the eruption had implicated the nose, both cheeks, lip, the upper arm and elbow, and had formed a semicircle on chest and back. The arm was contracted at the elbow joint. On the affected parts prominent yellowish scales were seen, which covered ulcers, roundish in the early stages and later confluent. The fundus was formed by raised reddish granulation tissue. In between these scabs was, here and there, newly formed glossy skin.

In another case (fig. 8), the nose was partly destroyed by the specific granuloma, which spread from the nose to the cheek.

Yaws was as prevalent amongst the civilised natives as amongst those who had hardly come under the Government influence, and had had no intercommunication with the neighbouring tribes.



### VENEREAL DISEASES

It is very difficult indeed to map out the distribution of venereal disease amongst natives during a journey from village to village. Natives are prone to hide venereal disease from each other, and systematic inspection, especially of women, is practically impossible. The diagnosis of 'Syphilis' in a native community is more difficult than in Europeans, on account of the occurrence of various ulcers and after effects of ulcers, which closely resemble the syphilitic lesions. Many of the manifestations of the tertiary or late stage of yaws, as described by Castellani, are identical with those of tertiary syphilis.

From the limited observations made, syphilis has not spread to any extent amongst the natives in the coastal districts of British New Guinea. This is in all probability due to the strict measures adopted by the Government against its spread. Lock hospitals have been established in different districts, and all cases reported are interned and only discharged after thorough treatment.

A scanty number of isolated cases were found here and there, but only in those districts where the natives had come in close contact with Europeans.

### ULCERATIVE GRANULOMA

A few cases of ulcerative granuloma were seen in the eastern parts of New Guinea, several of them under the care of Dr. Giblin in the Lock Hospital at Samarai. Only three typical cases were encountered in natives living in villages on the east coast, and a few sporadic cases in the western districts.

### GONORRHOEA

Cases of gonorrhoea may be found in all localities where the natives have come in contact with Europeans. In several villages in the Goaribari district, where communism is still existent, from a third to a half of the population were suffering from the typical lesions due to *Gonococcus*. The clinical symptoms did not differ in severity from those observed in a temperate climate, although venereal sores were more frequent on account of the lack of personal cleanliness.

**A PECULIAR DISEASE CHARACTERISED BY ARTHRITIS,  
OSTEITIS AND PERIOSTITIS AMONGST NEW GUINEA  
NATIVES**

In his article on skin disease in the tropics, Plehn (1914) mentions that Castellani, Howard, Schüffner and others have attributed joint affections and periostitis to yaws, regarding them as its tertiary stage. He also states that he himself observed similar bone lesions in Cameroon natives, which he considered as the final result of chronic osteomyelitis. Skiagrams, appended to the publication, show thickening of the periosteum of the bones of arm and hand in children.

A disease characterised by similar symptoms was encountered amongst New Guinea natives of different ages, of whom, in several instances, fairly reliable histories could be obtained.

The distribution of these cases was irregular, many villages being quite free, whilst others furnished six or more cases, most of which failed to show any signs of previous attacks of yaws. No evidence could be gathered as to a connection between the incidence of these two diseases.

It would be premature to state or deny definitely any relationship between yaws and the disease in question, until cases have been observed, where the two merge into each other without any prolonged interval. The irregular distribution, the similarity of the clinical histories, its absence from tropical regions where framboesia is very prevalent, are points in favour of the supposition that the two complaints are separate clinical entities.

This disease is exceedingly chronic, and is termed by the natives of the central district 'Roaki,' and by the natives of Kiwai Island 'Buuo' or 'Auma.'

The most prominent symptom is an affection of one or more of the joints, ranging from a hot painful swelling to suppuration and the formation of sores with fistulae in the skin above the joints, which discharge an amber-coloured clear liquid containing floccular particles. At the same time spindle-shaped thickenings of the larger and smaller bones of the extremities become noticeable, which, especially when the small bones of the hand and foot are implicated, may lead to softening and resorption of the bone. Similar sores to

those above the joints may make their appearance above the swellings of the bone.

The disease affects natives of all ages, men, women and children, although the majority were young persons. The youngest patient was about eight years of age.

As far as could be ascertained, the disease begins with an affection of one or more joints, without any special predilection being noticed. The joints swell up and become painful. After a varying period the swelling may abate or increase, and in this case the pain prevents the patient from using the affected extremity. After a prolonged period fistulous openings may appear in the neighbourhood of the joints secreting at first flocculent pus, and later on an amber-coloured clear fluid.

The foot so affected (Plate XXI, fig. 9) closely resembles Madura foot without the presence of the typical granules in the pus.

In other cases the bones may become affected, at first either in close proximity to the joint or the diaphysis of the long bones, mostly the tibia, ulna or radius; or in still other cases the metatarsal and metapthalangeal bones may show the change. The bone thickens, and a spindle-shaped swelling becomes noticeable which is not painful on pressure. This thickening is so well marked that it can readily be noticed on inspection (see figs. 10, 11).

These swellings of the various bones may remain unaltered for years, even for the rest of the patient's life-time. In other cases, especially when the smaller bones of hand and foot are the seat of the swelling, the lesion clinically resembles osteomyelitis. Fistulous openings appear which discharge copiously at first, and in time the whole of the affected bone may become resorbed. This process leads to peculiar disfigurement of the feet and hands (fig. 12). In cases where one of the metacarpal or metatarsal bones is resorbed, the fingers or toes become either retracted or even displaced, as in Plate XXII, fig. 13, where the large toe is in an abducted and hyper-extended position.

The frequent occurrence of the swelling of the radial, ulnar and tibial bones simultaneously with swellings of the metacarpi or metatarsi and swellings of the joints, appeared to furnish proof that all these lesions were due to the same disease.

The lesions of the hand and feet referred to may easily give rise

to a mistaken diagnosis. More than one case on superficial examination resembled nerve leprosy. A thorough examination, however, excluded this diagnosis, since in every case the finger tips were normal, the nails still well formed, and the thickening of the main nerve trunks was absent.

In a later stage ulcers are formed in the skin over the affected joints and bones. These are superficial, of varying size up to 25 cm. in length, oblong in shape with irregular edges; the granulation tissue is only slightly raised, of dark reddish colour, discharging an amber-coloured clear fluid. On the whole they show a tendency to spontaneous healing, forming dense scar tissue (fig. 14).

It was impossible to obtain any conclusive evidence as to the sequence of the lesions of bone and skin, since the information obtained from intelligent natives varied considerably. Some of them pointed out that the bone and skin lesions arose independently, others, however, stated definitely that the bone lesion preceded the skin lesion.

Several of the cases resembled, clinically, osteomyelitis with fistulous openings. In the majority, however, an inserted probe did not reach the bone.

The histological examination of the granulation tissue of one case did not show any peculiarities. It resembled, microscopically, typical granulation tissue containing a number of phagocytes and numerous bacteria and cocci on the surface.

Short case histories so far as obtained, and a clinical description of a few typical cases, may be helpful to illustrate this interesting and somewhat obscure disease.

*Case 1. Idiri—Woman about 35 years of age.*

The patient had been ill for a long time, perhaps five years. She never suffered from any painful joint lesions. The first symptom noticed was a hard swelling of the tibia. A short time afterwards sores formed above the swelling of the bones, which healed up after a long time. The metatarsus of the big toe of her right foot began to swell, and afterwards small openings appeared in the skin above the swelling, discharging, for a long time, thick matter. A considerable time afterwards these fistulae ceased to discharge, and healed up with displacement of the large toe.

At the time of examination the shaft of the right tibia showed a considerable spindle-shaped swelling. Numerous scars had formed in the skin above and on the dorsal side of the foot. The metatarsus of the big toe had disappeared, and the toe consequently was abducted and hyperextended. The right wrist joint was swollen and painful, the ulna showed in the middle of its shaft a marked spindle-shaped swelling, about 10 cm. long.

*Case II.* Boy about 12 years of age. Comp. fig. 11.

The boy had been ill since childhood, and the disease had begun with painful swellings of the bone. Some time afterwards large, slowly healing sores formed in the skin above the swellings.

The boy was very emaciated. Both tibiae were curved forward, the bone thickened and the skin above showed ulceration. Both ulnar bones had, in their upper third, a spindle-shaped thickening with a fistulous opening, on one side discharging liquid pus. There was extensive ulceration on both the arms, where roundish irregular islets of reddish, raised granulation tissue were surrounded by very thin newly-formed skin. Both legs were ankylosed at the knee joint.

*Case III.* Small boy about 8 years of age.

The patient was hale and hearty till about one year ago, when his right ankle joint began to swell up. Shortly afterwards his right knee joint became painful and swollen, and later the left knee and both wrist joints became affected in the same way. When examined the boy was considerably emaciated, both the knee and wrist joints were swollen and painful. The muscles of both calves as well as the muscles of both forearms were atrophic. The distal end of both of the radial bones was considerably enlarged.

The patient did not show any sign of present or past infection with yaws, and throughout the village not a single case of yaws was seen.

*Case IV.* Man about 35 years of age.

According to information obtained from the patient, the disease had started about twenty years ago with pains in his back and in the joints of his arms and legs.

When examined his right foot was very much swollen, and all

the toes of his foot had dropped off as the result of ulceration. The skin of the foot was uneven, lumpy, and numerous fistulous openings were seen, some apparently closed, others secreting on pressure whitish floccular masses. The muscles of the leg itself were atrophic, and the skin above the much thickened tibiae covered with old scars. The left tibia showed the same changes, simulating a chronic oostomyelitis. The left elbow joint was swollen, hot and painful, and the tip of the elbow the seat of a small ulceration.

The fingers of the left hand were deformed, the metacarpophalangeal joint of the fourth and fifth finger were ankylosed. The shafts of both radial bones showed a spindle-shaped thickening, and there were deep scars over both radio-carpal joints.

### ULCERS IN THE TROPICS

A great variety of superficial and phagedaenic ulcers, apparently of the most varied etiologies, are collected under the name 'Tropical Ulcer.' The clinical differentiation of ulcers in the tropics becomes still more involved on taking into consideration the careless habits and the lack of cleanliness amongst native races in general. Any superficial skin abrasion on hand or leg, which if at all cared for would heal, may give rise to a small ulcer which on account of the continuous irritation caused by sand, dirt and flies may spread. It is probable that many of the large phagedaenic ulcers are not due to one specific microorganism, but are caused by a variety of microorganisms invading a small wound due to an accident.

The observations made in British New Guinea appear to bear out this statement. On the whole the incidence of ulcers depends largely on the state of cleanliness, which is greatly influenced by the natural conditions and surroundings of the different villages. In the districts west of Orokolo, where the villages are built on high piles on muddy grounds, and where fresh water is very scarce, ulcers of varying types are encountered in great numbers among the natives.

The natives in these districts, where sago palms grow in great abundance, use sago as their staple article of diet, and spend, in consequence, much of their time in preparing sago from the trunk of the palm. The sago palm carries large thorns on the rib of the

leaves and on the trunk, and the natives are often injured by them when felling the trees. The wounds in the majority of cases are not cared for, and undoubtedly many ulcers are originally due to wounds caused by these thorns.

The number of inhabitants with ulcers in villages built on dry, sandy soil is much smaller.

The hypothesis that many of these ulcers are caused through secondary contamination of smaller or larger injuries of the skin receives additional support from the experience gained in their treatment. A great number of the sores heal up readily under the application of antiseptic powders only, without any specific treatment. It must, however, be kept in mind that the scar, covered by a newly-formed skin on the original site of the ulcer, is always more liable to injury and may again become the seat of a similar ulcer.

The question of the differentiation and diagnosis of ulcers becomes more complicated by the facts that in the majority of the natives the ulcers are in an advanced stage, that it is impossible to obtain a definite and reliable history, and that advanced ulcers of many different etiologies are similar. The question of a tertiary form of yaws is still under discussion, and the clinical symptoms attributed by various observers to tertiary yaws may be due to other factors.

The diagnosis of syphilis is not less complicated, and a traveller with little experience of natives in their original state might make the diagnosis of syphilis where its occurrence may be excluded with certainty.

Gangosa, so similar to tertiary syphilis in its advanced stages, causes ulcers of arms and legs which, when far advanced, are in no way characteristic.

Many other complaints, such as leprosy, various skin diseases, etc., may take an atypical form in natives and make a differential diagnosis in the late stages impossible.

Histological examination of the granulation tissue does not permit a definite differentiation of the ulcers, since the microscopic appearance of sections of different and clinically apparently well characterised sores is similar. Smears taken from the surface are, as a rule, a pell-mell of saprophytic bacteria and yeasts of various

sizes and shapes, whilst smears taken from the deeper parts generally do not show any bacteria.

The examination of the natives of the south coast of British New Guinea revealed the presence of a great number of ulcers. Several were well characterised and could be classified, whilst others did not show any typical features.

#### **TROPICAL SLOUGHING PHAGEDAENA, ULCUS TROPICUM**

*Ulcus tropicum*, a complaint occurring in practically every part of the tropics, is known under nearly as many names as there are localities where it has been found.

Clinically it is a chronic sloughing ulceration of phagedaenic character, which may spread into the depth, laying bare muscles and bones and showing a tendency to spontaneous healing, or leading, if neglected, to considerable deformities of the affected extremities.

The literature on tropical sore is fairly extensive, and has been summarised by Plehn (1914). It is, however, beyond doubt that several varieties of sores, specific and non-specific, have been previously included under the same heading.

Prowazek (1907) described a spirochaete, *Spirochaeta schaudinni*, as the specific cause of the disease. This spirochaete is actively motile, resembling the balanitis spirochaete, possessing only rarely periplast on one end; it multiplies by longitudinal fission and forms involution forms.

The same spirochaete was found by Keysselitz and Mayer (1909) in smears taken from the deep layers of ulcers, and by Wolbach and Todd (1912); although the latter authors consider that 'The fact that these ulcers are almost invariably found solitary and on regions exposed to trauma supports the explanation that the process is a reaction to an organism having slight power of invasion.'

Le Dantec (1911) had previously pointed out that two conditions were essential for the development of a tropical sore, namely an abrasion of the skin and exposure of this to soiling by humid earth.

A direct experimental transmission of the sore to animals such as guinea-pigs, monkeys, and apes and to men has been unsuccessful in the hands of all observers. Blanchard (1914) succeeded in transmitting a tropical ulcer from man to man, but



only after having produced at first an artificial necrosis of the skin, which was some days afterwards inoculated with the granulation tissue from a case of tropical ulcer.

Cases of tropical ulcers were found in varying numbers, throughout the coastal districts of British New Guinea, and barely one village was entirely free from this complaint.

In the villages situated on high, dry ground only a small number of cases was seen, whereas in the villages in the western parts of New Guinea, which are built on swampy, muddy ground, a great number of cases was encountered. It seems, therefore, that the frequency of tropical ulcers stands in some relation to the incidence of mangrove mud.

The tropical ulcer of New Guinea does not differ essentially from that observed in other parts of the tropics. It occurs mostly on the lower extremities, on the dorsal surface of the foot, in the neighbourhood of the external and internal malleolus, on the skin, rarely on the back of the thigh. In the early stages the ulcer resembles a boil. There is an ulcer in the centre which is covered by a scab, slightly raised above the surface of the skin, showing fluctuation and a certain amount of inflammatory reaction around. Very soon, if neglected, this small round ulcer begins to spread, the epithelial layer breaks down, showing underneath a fungating ulcer, which bleeds very readily and is covered with a small amount of thick greyish pus. The ulcer is well defined from the surrounding tissues.

In the beginning the ulcer spreads very rapidly, is irregular in outline, and covered by a layer of foul smelling greyish semi-fluid pus, which forms a pseudo-membrane covering the dirty greyish to reddish granulations (fig. 15). After a varying interval the ulcer spreads into the depths, destroying the subcutaneous tissue and the muscles (fig. 16) and often attacking the underlying bone, and gives rise to periostitis and osteitis. Now and again a true osteomyelitis may follow the ulcer, and then pieces of necrotic bone are often found embedded in the granulation tissue. In other cases, however, the ulcer remains superficial and the granulation tissue proliferates and projects above the level of the surrounding skin. The borders in chronic ulcers are either sharply defined or sometimes undermined. The whole of the foot may swell up, resembling elephantiasis (see Plate XXIII, fig. 17).

The ulcers are very chronic, and may persist for many years without endangering the life or general health of the patients. Judging by the number of natives seen with extensive scars above the tibia and with thickening of the bone, it is evident that even large ulcers of long standing may suddenly begin to heal up. At other times the patients emaciate considerably, become completely crippled, and consequently are confined to their houses and die of the effects of septic resorption.

*Treatment.* Salvarsan has been introduced within the last few years for the treatment of tropical ulcer with excellent results. This mode of treatment is, however, too expensive and complicated to be generally adopted. The thorough removal of the granulation tissue with a sharp spoon, followed by careful dressing with a mixture of iodoform-boracic acid in the proportion of 1 to 10 is efficient. The majority of the ulcers yield readily to this treatment, and even large sores may heal up within a few months.

Natives who have been in contact with Europeans for a longer period bandage the sores with banana-leaves, and this treatment seems to be fairly successful.

#### *Pathological Histology of Ulcus tropicum*

Opportunity was taken to obtain granulation tissue of 10 cases suffering from *Ulcus tropicum* in various stages for further histological examination. The tissue which was preserved in 80 per cent. alcohol was embedded in paraffin and sections stained with haematoxylin eosin, Breinl's saffranin-methylene blue, orange-tannin (Breinl, 1908), and by Heidenhain's method, using iodine-potassium iodide solution as a mordant.

The results of histological examination were, on the whole, in accordance with those published by Keysselitz and Mayer and by Wolbach and Todd.

The sections of the granulations from the centre of the lesions consisted of a loose fibrous mesh-work, the meshes were filled with exudate, containing red blood corpuscles, leucocytes and lymphocytes, plasma-cells, and a few eosinophile leucocytes were scattered here and there. The granulation tissue was richly vascularized, many of the arteries showing endarteritis obliterans, and was covered by a loose layer of fibrin, which contained erythrocytes in all stages

of disintegration, lymphocytes, cell detritus and bacilli and spirochaetes in great numbers.

The sections from the border of the ulcer showed a marked hypertrophy of the epithelial layer, being of many times its normal thickness. In between the epithelial cells were roundish or oval well-defined spaces of varying size, filled with a loose and oedematous fibrous tissue, in which a small number of polymorpho-nuclear leucocytes were embedded. The epithelial strands between these spaces became thinner, the cells showing degenerative changes, increasing in size and becoming vacuolated. The nuclei became irregular in shape and finally disappeared. In close proximity to the ulcers these spaces were densely packed with leucocytes and lymphocytes, resembling microscopically small abscesses.

The superficial epithelial cells from the edge of the sore showed degeneration, the cytoplasm was vacuolated, and the nuclei were irregular and did not stain evenly.

In the sections the same microorganisms as described by previous observers were found. Fusiform bacilli occurred mostly on the surface of the ulcer, and were found in abundance in between large numbers of bacteria and cocci.

The spirochaetes—*Spirochaeta schaudinni*, Prowazek—to which the etiology of *ulcus tropicum* is generally attributed, were seen in small numbers in sections of several of the cases, mostly lying on the surface between the other microorganisms or in between the epithelial cells adjoining the ulcer.

### CONTRACTING SORE

A special type of ulcer was encountered, cases of which were irregularly distributed throughout the coastal districts of British New Guinea. A comparatively greater number of cases was seen in the eastern parts, but sporadic cases were discovered in practically every district visited. This ulcer differed in its appearance considerably from other sores, and its clinical features were so well marked and uniform that it was considered a hitherto undescribed form of skin ulceration. It was named 'Contracting Sore' on account of its effects. It heals up with formation of dense scar tissue, leading to contraction of the joints.

The sore appeared in the majority of cases in the neighbourhood of joints, for example on the inner or outer surface of the knee (fig. 18), on the dorsal surface of the foot, above the tendo achillis, or in the neighbourhood of the shoulder joints (fig. 19). Other localities, such as around the anus (fig. 20), the abdomen or chest or arms were only rarely the seat of the ulcer. On the whole it occurred more frequently in young persons, and was often found in children. Natives of more mature age only showed the typical scar formations.

The earliest stage was in a boy about 10 years of age. A small ulcer of about 3 mm. in diameter was found on the inner side of the knee. It was round and possessed irregular burrowing edges; the surface was covered by a dry looking, slightly raised, yellowish, greasy scab; the surrounding tissue did not show any marked inflammation. Underneath this scab, which could easily be removed as a whole, was a collection of thick greyish pus accumulated above the granulating surface, which was slightly below the level of the skin, of reddish colour, even and of the appearance of coarse sandpaper. The granulation tissue was firm and could easily be scraped off in strips by means of a sharp spoon.

The ulcers spread slowly and gradually, at first remaining roundish, but after a varying period, becoming irregular in shape, and were always covered by the same kind of yellowish-grey scab, secreting from the edges an amber-coloured yellowish clear fluid. They never spread below the underlying fascia, never attacked the bone, and the granulation tissue could always be taken off in whole strips, forming a layer of 2 to 5 mm. in depth and exhaling a pungent, penetrating odour.

The ulcer may implicate in some natives a small area only and the process may come to a standstill. More often extensive areas may be affected, as in fig. 21, where the sore had spread over the whole flexor surface of one leg.

At the time when the ulcerative process is still progressing there may be in parts a marked tendency to spontaneous healing (see Plate XXIV, fig. 22). The granulating surfaces clear up and are replaced by dense and hard scar tissue, possessing a smooth surface, and being often extensively intersected by slightly raised strands of hard connective tissue. After a varying period, ranging from six

months to three to four years, the ulcer may completely heal up in the way described, giving rise to contraction of the affected parts closely resembling at first sight the scar formation due to deep burns (see figs. 23, 24).

This contracture invariably causes further deformities (fig. 25), such as complete ankylosis of the joints and wasting of the muscles on account of inactivity.

*Histology.* From nine cases suffering from different stages of 'Contracting Sore' granulation tissue was obtained by scraping the sore with a sharp spoon. It was fixed in 80 per cent. alcohol and sections made and stained by various methods. The granulation tissue from an early ulcer about 3 cm. in diameter showed, on microscopic sections, a marked hypertrophy of the epithelial layer immediately surrounding the ulcer. These hypertrophied layers of epithelial cells were interspersed with irregular spaces filled with a loose oedematous connective tissue, the meshes of which contained a small number of leucocytes and lymphocytes and a few plasma cells; the nearer to the ulcer the larger were these spaces, the thinner the epithelial strand separating them and the denser the cell infiltration.

The corium was oedematous and contained a certain amount of infiltration of lymphocytes, leucocytes and plasma cells, which infiltration was denser around the blood vessels.

In the central parts of the ulcer the epithelial layer had been replaced by granulation tissue, showing here and there a few epithelial cells in varying stages of degeneration. The granulation tissue itself did not show any distinctive features.

A number of large cocci and bacteria were seen in sections from the surface of the ulcer lying between red blood corpuscles in different stages of disintegration and coagulated fibrin. There were neither fusiform bacilli nor spirochaetes in the surface layers. In the deepest layer of the epithelium, however, in the rete mucosum of the malpighian layer especially in the cells lining the spaces referred to, a great number of spirochaetes were found, which were either intracellular or were lying in the fine clefts which separated the cells. These spirochaetes occurred either singly or sometimes in bunches, and stained readily with iron haematoxylin after iodine-potassium iodide had been used as a mordant. The spirochaetes were found

only in this epithelial layer, and were neither present in the granulation tissue nor in the discharge, of which a small number of smears was examined.

The sections of the granulation tissue of advanced cases did not show any especial features. It was richly vascularized, some of the vessels showing endarteritis obliterans. There were numerous leucocytes and lymphocytes, a few plasma cells and many phagocytes containing small roundish cell inclusions which stained blackish with Heidenhain's iron haematoxylin, and orange when Breinl's method was employed.

The deepest layers of the granulation tissue consisted of a meshwork of newly-formed dense connective tissue, with numerous fibroblasts with a small amount of small-celled infiltration.

Judging from the histological examination, the ulcer begins with a progressive destruction of the epithelial covering of the skin, whilst the cells of the deepest layers of the rete mucosum are invaded by numbers of spirochaetes. The deep layers of the granulation tissue show a tendency to formation of a dense connective tissue.

On the whole the granulation tissue of these contracting sores does not differ microscopically in any essential point from that of the *ulcus tropicum*. The small-celled infiltration is much denser in the latter, the surface layers contain a great number of microorganisms, fusiform bacilli, bacteria and spirochaetes, whereas in the sections of the contracting sore only a very small number of bacilli were found in the surface layers, and the spirochaetes were only seen in and between the epithelial cells of the rete malpighi.

The histological examination of the granulation tissue of the contracting sore did not reveal any microorganism which could be regarded as specific.

The presence of spirochaetes in numbers in and between the layers of the epithelial cells of the malpighian layer is very suggestive, but spirochaetes are found in great numbers in many and various ulcers of the skin. Moreover, it was impossible to work out any detailed morphology of the spirochaete with the material collected.

### ULCUS INTERDIGITALE DESTRUENS

A form of ulceration closely resembling 'Ulcus interdigitale' as described by Castellani and Chalmers (1913) was seen among the natives living in the swampy parts of the western New Guinea.

According to these authors, the ulcer begins as a fissure in between the toes, which 'Rapidly deepens and enlarges into a large oval ulcer with a dull dark red fundus and sodden-looking margins. There is practically no discharge whatsoever.'

The sores observed resembled, in some respects, those described. The ulceration usually started as a fissure between the toes, or more often in the sulcus below the toe (Pl. XXV, fig. 26). This small fissure formed, after a time, into a small painful ulcer, and spread fairly rapidly upwards to the toe and downwards to the sole of the foot (fig. 27). The ulcer is deep, possessing sharp irregular edges, and the granulation tissue, which is covered by an irregular dirty greyish scab, has a reddish uneven fundus and discharges a great deal of thick yellowish pus. When the ulceration spreads upwards in between the toes it causes, on healing, the two adjoining surfaces of the toes to grow together. Sometimes the whole toe becomes covered with granulation tissue which leads to complete loss of the affected toe. Such an example is shown in fig. 28, where the ulceration has spread to the sole of the foot, implicating the ball of the foot, and had given rise to a deep, irregular granulating sore, and two of the toes had been amputated by the ulceration.

These interdigital sores are very chronic, and show, after a shorter or longer period, a tendency to spontaneous healing after having caused considerable deformity of the foot. Fig. 29 was taken from a case where the fourth and fifth toes had disappeared, and where the second and third toes had grown together on their adjoining surfaces.

Cases of this type of ulcer were widely distributed throughout the districts of New Guinea west of Orokolo, and affected mostly men. The ulcers differ from the 'Ulcus interdigitale' on account of their copious discharge and their tendency to lead to the destruction of the affected toes, and the name 'Ulcus interdigitale destruens' is proposed for them.

*Pathological Histology.* From two cases suffering from inter-

digital sores, granulation tissue was obtained by scraping the sore with a sharp spoon.

Microscopically the sections did not show any characteristic features. The epithelial surrounding of the ulcer was hypertrophic, the granulation tissue which was richly vascularised showed a heavy leucocytic and lymphocytic infiltration. Numerous bacteria and large cocci occurred on the surface, lying in between fibrin cell detritus and disintegrating red blood corpuscles.

### GANGOSA

Gangosa or Rhinopharyngitis mutilans, a disease which causes ulceration and destruction of the nose, soft and hard palate, and of the skin of the face, is endemic in most of the coastal districts of British New Guinea. A full account is being published in this journal, describing *Cryptococcus mutilans* as its etiologic agent (see p. 213). In several far advanced cases the hands and feet were the seat of extensive ulceration, the fingers were enlarged, the back of the hands swollen and discharged copiously.

These lesions are in all probability due to the same etiologic agent as the lesions of the face.

### SKIN DISEASES

A great number of different skin diseases are prevalent amongst the natives of New Guinea.

*Tinea imbricata* (Plate XXVI, fig. 30) was found in every district, equally prevalent amongst those natives who had been civilised for years, and amongst those who had not come in contact with Europeans and had not been away from the district.

A few cases of *Leucoderma* were encountered, mostly confined to the hands (fig. 31).

Only two cases of true *Albinism* were found in the Trobriand Islands of the north-east coast of New Guinea—one was in an adult man, the other in a baby about three years of age.

A peculiar form of skin disease was encountered somewhat resembling *Acne* in its clinical appearance, and leading to destruction and peculiar scar formation of the affected parts. Sporadic cases of this complaint, occurring in men and women, were distributed



throughout British New Guinea. The nose, sometimes the upper lip, both cheeks and the forehead, were covered with pustules from which sebaceous material could be expressed. The nose became enlarged, hyperaemic, and the skin uneven and covered with warty excrescences. In one case the upper lip was similarly affected (fig. 32). Here and there were small patches of smooth, shiny scar tissue, and small roundish depressions with a reddish-looking fundus, resembling to some extent the pitting after smallpox (fig. 33).

This disease showed a tendency to destroy the affected parts. The free border of the alae was uneven and irregular and, in two out of eight cases, the soft palate and the uvula had been destroyed and replaced by dense scar tissue.

The skin of the body of the patients was clean and did not show any lesions whatsoever.

As far as histories could be obtained, the complaint seemed very chronic, extending over years, and invariably healed spontaneously. As far as could be ascertained, the disease made its first appearance in the form of pustules on the alae of the nose, whence the lesions spread gradually to the cheeks and upper lip.

An advanced active case is of typical appearance, but when the lesions had partly healed the differential diagnosis now and again offered difficulties.

The microscopic examination of the sebaceous material expressed from pustules did not give any clue to the etiology.

#### CONCLUDING REMARKS

The material collected during the journey has shown that a great many diseases which occur commonly amongst the native populations in other parts of the tropics are present also in New Guinea.

Of epidemic diseases only dysentery has made its appearance within the last twenty years, and since 1896 outbreaks have been recorded from different districts of the Possession.

As the primary object of the expedition was the mapping out of the distribution of diseases in New Guinea, the short stay did not permit a thorough investigation into a number of complaints, especially ulcers and skin diseases, many of which could not be

diagnosed and might, on further examination, prove new to medicine.

The two expeditions were made possible by the practical interest of the Department of External Affairs, Commonwealth of Australia.

I take the opportunity of expressing my indebtedness for courtesy and assistance received from the Secretary of the Department of External Affairs, Mr. Atlee Hunt, C.M.G., from the Lieutenant-Governor of Papua, Judge J. H. P. Murray, C.M.G., and his personal staff, and from the Government Secretary, Mr. A. M. Campbell.

## APPENDIX

## LIST OF PAPUAN MOSQUITOS

- \**Anopheles (Myzorbhynchus) barbirostris*, var. *bancrofti*, Giles.
- Anopheles (Nyssorbhynchus) annulipes*, Walker.
- Anopheles (Cellia) punctulata*, Dönitz-Theobald.
- \**Armigeres obturbans*, Walker.
- Neosquamomyia breinli*, Taylor.
- \**Stegomyia fasciata*, Fabr.
- Stegomyia scutellaris*, Walker.
- Stegomyia pseudoscutellaris*, Theobald.
- Stegomyia ornata*, Taylor.
- Stegomyia atra*, Taylor.
- Scutomyia notoscripta*, Skuse.
- Lepidotomyia lineatus*, Taylor.
- Leucomyia australiensis*, var. *papuensis*, Taylor.
- Leucomyia ? albitarsis*, Taylor.
- Culicelsa vigilax*, Skuse.
- Culex sitiens*, var. *milni*, Taylor.
- Culex fatigans*, Wied.
- Pseudotaeniorhynchus conopas*, var. *giblini*, Taylor.
- Chrysoconops brevicellulus*, Theobald.
- Taeniorhynchus septempunctata*, Theobald.
- Taeniorhynchus uniformis*, Theobald.
- Taeniorhynchus papuensis*, Taylor.
- Melanoconion papuensis*, Taylor.
- Finlaya poicilia*, Theobald.
- Uranotaenia nigerrima*, Taylor.
- Hodgesia triangulatus*, Taylor.

The bulk of the mosquitos, of which a list has been prepared by Mr. F. H Taylor, were collected by Dr. Giblin and the writer at the Lakekamu gold field, except those marked with an asterisk, which are previously recorded species. (Taylor, 1914).

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EXPLANATION OF PLATES

PLATE XIX

- Fig. 1. Elephantiasis of scrotum.
- Fig. 2. Elephantiasis of vulva.
- Fig. 3. Leprosy; advanced case.
- Fig. 4. Juxta-articular nodules.



FIG. 1



FIG. 3



FIG. 2



FIG. 4







PLATE XX

- Fig 5. Juxta-articular nodules.
- Fig. 6. Yaws. General eruption. Secondary stage.
- Fig. 7. Yaws. General eruption. The same case as fig. 6.
- Fig. 8. Yaws. Note destruction of nose.

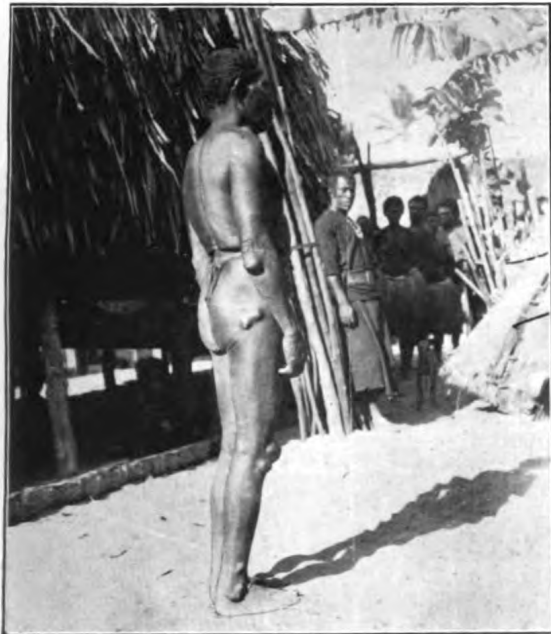


FIG. 5



FIG. 6



FIG. 7

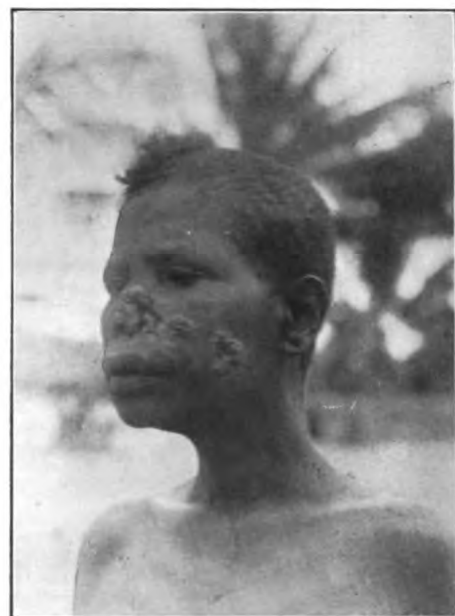


FIG. 8





PLATE XXI

Figs. 9-12. Disease characterised by osteitis, periostitis, arthritis, and formation of sores.

Fig. 9. Late stage, resembling Madura foot.

Fig. 10. Swelling of bone affecting the tibia.

Fig. 11. Swelling of bone affecting the diaphysis of radius.

Fig. 12. Mutilation of hand after resorption of metacarpal bones.

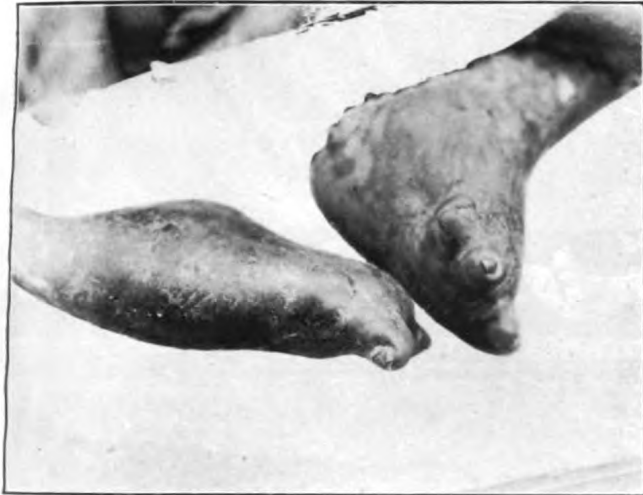


FIG. 9



FIG. 10

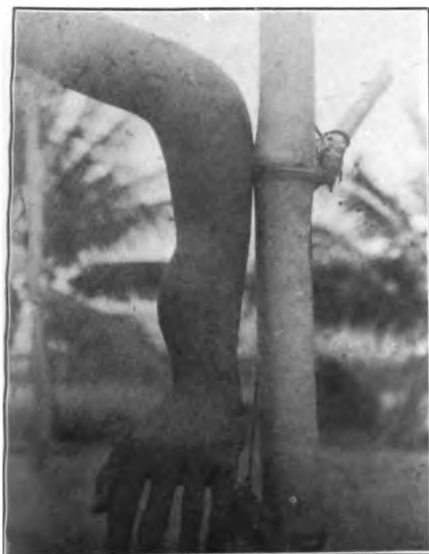


FIG. 11



FIG. 12







PLATE XXII

Figs. 13-14. Disease characterised by osteitis, periostitis, arthritis, and formation of sores.

Fig. 13. Metatarsus of large toe disappeared, the toe in hyper-extension and abduction.

Fig. 14. Late stage of disease, showing irregular scar formation.

Figs. 15-16. Ulcus tropicum.



FIG. 13



FIG. 14



FIG. 15



FIG. 16







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PLATE XXIII

Fig. 17. *Ulcus tropicum*.

Figs. 18-21. Contracting sore.





FIG. 17



FIG. 18



FIG. 19



FIG. 21



FIG. 20





PLATE XXIV

Figs. 22-25. Contracting sore.



FIG. 22



FIG. 23



FIG. 24



FIG. 25



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PLATE XXV

Figs. 26-29. Ulcus interdigitale destruens.





FIG. 26



FIG. 28



FIG. 27



FIG. 29







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PLATE XXVI

Fig. 30. *Tinea imbricata*.

Fig. 31. Leucoderma.

Figs. 32-33. Skin disease resembling Acne.



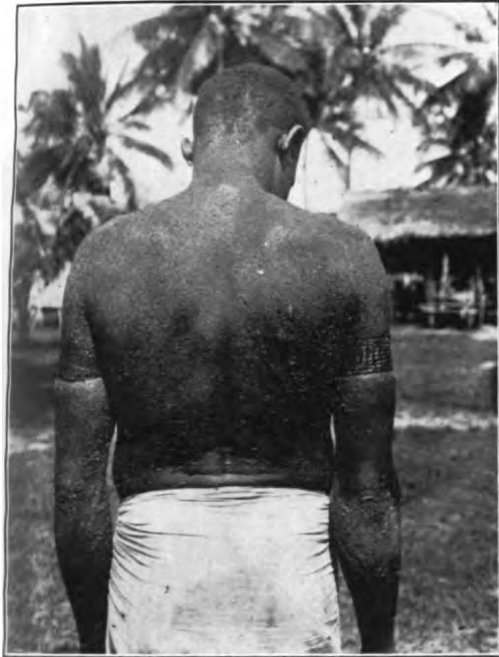


FIG. 30

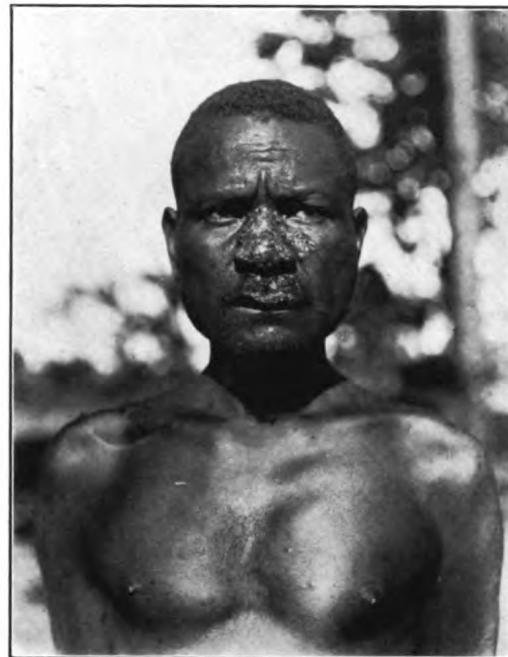


FIG. 32



FIG. 31

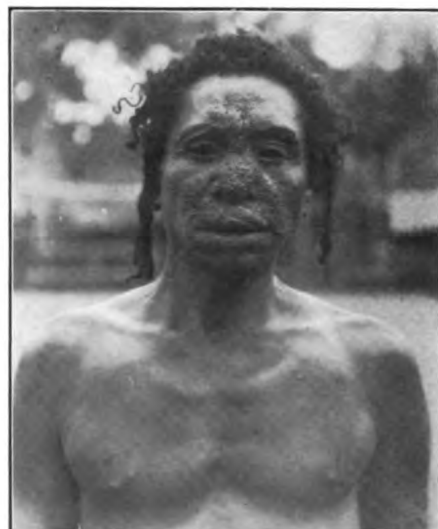


FIG. 33

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# INSECT FLAGELLATES AND THE EVOLUTION OF DISEASE, WITH REMARKS ON THE IMPORTANCE OF COMPARATIVE METHODS IN THE STUDY OF PROTOZOOLOGY

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## I. INTRODUCTION

The parasitic theory of disease has been greatly extended and consolidated during the last generation. In the domain of bacteriology it has perhaps become best known, at any rate to the general public. Vast strides have been made during the latter half of the period in our knowledge of animal parasites, especially of the inter-relationship of Protozoa and arthropods in the propagation of disease. There is also a re-awakening as to the importance of mycology, or the study of fungoid organisms as pathogenic agents of economic importance.

In the domain of protozoology, with which the present paper is concerned, the progress, although rapid, has not been so great as it might have been. Some of the reasons for this are that the work

is in the main in the hands of medical men whose knowledge of zoology is generally of the slightest and their acquaintance with the comparative aspect of the subject just as limited, while the few zoologists engaged have in some cases drifted into a narrow outlook and become lost in academic terminologies and discussions. The result is that though many important discoveries have been made, they have not reached full fruition, chiefly on account of lack of application of the comparative method. As an example, the parasite of kala-azar may be considered. The causal organism, *Leishmania donovani*, was described in 1903 by Leishman and by Donovan, while Rogers in 1904 cultivated it and discovered the herpetomonad stage. The latter discovery, that of Rogers, was of fundamental importance, but its application is, even to-day, only realised in varying degrees by two or three British and French workers. However, the output of literature on kala-azar and other leishmaniasis is simply enormous, though the progress resulting therefrom is very slight, because of the overburdening amount of controversy obscuring the issue. When Rogers in 1905 suggested the bed-bug as the probable transmitter of kala-azar in India, and Patton began to work at the same, the results obtained were largely buried in criticism, much of it speculative and irrelevant. We read in 1912 such statements as the following: 'It is quite clear that the gut of the bug when it contains blood acts as a culture-tube in which various organisms can live and develop. . . . Hence the fact that the leishmania of kala-azar and oriental sore become flagellates in the bug's gut is in itself no proof whatever that these insects are the transmitters of these diseases.' It is to be regretted that those putting forward or supporting the latter remarks did not more carefully consider the suggestions and inferences to be drawn from cultural methods, and proceed forthwith to experiment directly with the herpetomonads found in insects, especially after Patton (1907) had given the first account of the complete life-cycle of such a herpetomonad, and Darling (1910) had suggested that a case of oriental sore in Panama had resulted from 'an inoculation with an invertebrate gut flagellate (*Crithidia* ?).' Again, we had the flood-gates of destructive criticism opened when Basile (1910-11) suggested that *Leishmania infantum* was transmitted by fleas, though it does not seem to have occurred to

any of the critics to begin experiments on the effects of flagellates of fleas when introduced into the vertebrates they infest. However, it is unnecessary to labour the point further, it is merely symptomatic of the age, pointing to the partial failure of the modern educational system, in that it tends to the development of trifling academic debate, nearly always useless and sometimes even harmful, and to a fostering of the partisan spirit, wherein a broad comparative outlook is seldom attained. Legitimate criticism is always welcome, but when it degenerates into mere retort and negation it is subversive of progress.

There is yet another branch of the study of pathogenic organisms which has not yet received attention, namely, the biological evolution of such forms as disease producers, and a consideration of 'disease in the making.' For this study a knowledge of comparative morphology and of the inferences to be made therefrom is absolutely essential, and any one who aspires to become a parasitologist must be prepared to undertake such a study.

## II. THE SIGNIFICANCE OF THE HERPETOMONAD STAGE OF *LEISHMANIA*

When Rogers in 1904-05 cultivated the Leishman-Donovan body, obtaining a herpetomonad flagellate therefrom, and suggested that the genus *Leishmania* should give place to *Herpetomonas*, a great step forward might and should have been made in the application of the idea, especially as Christophers (1904) in *L. donovani*, and Mesnil and colleagues (1904) in *L. tropica*, had shown that a structure, now known to be the root of the flagellum, could be found in some of the parasites.\* Patton (1908) also placed *Leishmania donovani* in the genus *Herpetomonas*. It was also pointed out that the natural herpetomonads of insects might be mistaken for developing *Leishmania*. However, these advances met with the inevitable destructive criticism, for as recently as 1912 we read: 'The genus *Herpetomonas* includes flagellates which have only one, and that an invertebrate host. The fact that the parasite of kala-azar has two hosts and can live and multiply in the organs of a vertebrate shows it to be profoundly

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\* Novy (1909) showed that a rhizoplast occurred also in *L. infantum*. (*Bull. Soc. Path. Exot.*, II, pp. 385-387.)

different from the true *Herpetomonas*, which lives only in the invertebrate. This fact alone would be sufficient to establish the genus *Leishmania*. . . . When a *Herpetomonas* becomes so changed that it has acquired the power of living and developing as an intracellular parasite in the body of a warm-blooded animal, and giving rise to such a serious disease as kala-azar, we are justified in concluding that it has passed out of the genus *Herpetomonas*, from which it originated, into the genus *Leishmania*. The pity is that the author, before penning these remarks, did not himself endeavour to determine experimentally whether *Herpetomonas* might not acquire the power of living and developing in a vertebrate, and that the record of the remarkable observations of Dutton and Todd, published in 1903, on the presence of herpetomonads in the blood of Gambian house mice was unknown to him, as to many others. I deeply regret the necessity for making these remarks; there is nothing personal in them, but the facts are there and cannot be gainsaid.

The successful cultivation of *L. tropica* and *L. infantum* again did not advance matters, but discussion arose as to the number of valid species of *Leishmania*, and numerous attempts were made to inoculate the viruses into laboratory mammals. Although expeditions were organised for the study of *Leishmania* in endemic areas, it never seems to have occurred to those working thereon to try to introduce herpetomonad flagellates of insects into vertebrates. Apparently, vertebrates, being thought much more powerful and important animals than invertebrates, must perforce be primarily considered and experimentation undertaken with them viewed as the principal hosts. Unfortunately, human suffering has paid dearly for this one-sided view, which is only equalled by the ridiculous arguments, that used to occupy the attention of amateur parasitologists and waste the time of learned societies, as to which was the primary or principal host of a digenetic parasite, was it the vertebrate or the invertebrate host? The answer is that each host has its own particular importance which must be carefully considered. In the case of the kala-azar parasite the pathogenic organism certainly seems to have arisen from a herpetomonad parasite of an invertebrate (probably an insect), and the flagellate has acquired the capacity to live within a vertebrate and so become digenetic.

Again, the intimate connection of natural canine leishmaniasis and of infantile kala-azar has been explained away by some. In other words, every step forward has been obscured by irrelevant criticism, and what should have been obvious inferences were either ignored or never given the opportunity of being put to the practical test. The comparative aspect was overlooked. The occurrence of natural herpetomonads in invertebrates must not only be acknowledged, but it must be allowed that they may become pathogenic when introduced into vertebrates.

### III. THE EXISTENCE OF THE HERPETOMONAD STAGE OF *LEISHMANIA* AND ALLIED PARASITES IN MAN

It is now known that Escomel in Peru in 1911 found the herpetomonad stage of *Leishmania tropica* in man, but seeming somewhat disturbed by his finding did not publish it at the time, nor realise its importance and significance. Monge, also in Peru, records (1914) the presence of the herpetomonad stage in dermo-mucosal leishmaniasis, and La Cava (1912) in *L. tropica* in Italy. Splendore (1912) saw elongating forms and a few flagellate stages in cases in Brazil.

Even before these observations, Darling (1906, 1909) in Panama had recorded a disease resembling kala-azar, and described the causal parasite therein as *Histoplasma capsulatum*. He stated that flagellate stages occurred in the lungs and spleen. As might be expected, the mention of the occurrence of flagellate stages passed almost unnoticed. In 1912 Rocha-Lima, working in Hamburg, stated that in his opinion *Histoplasma capsulatum* was a Blastomycete. Whether this be so or not, surprisingly little attention was paid to Darling's researches.

Again, in 1913 Franchini described a herpetomonad parasite from man, and called it *Haemocystozoön brasiliense*.

### IV. THE PRESENCE OF HERPETOMONADS IN NATURE IN OTHER VERTEBRATES

Dutton and Todd in 1903 recorded the presence of herpetomonad flagellates in the blood of mice in the Gambia. Unfortunately, these observations, first made in 1902, seem to have escaped general notice. They have since been interpreted otherwise by the surviving

author. Three Gambian house mice out of fourteen were found to be infected with flagellate herpetomonads, of which a clear description is given, together with dimensions. The infection was scanty, and the parasites were only seen in fresh preparations. The organisms were definitely compared with *Herpetomonas* (*Leptomonas*) *bütschlii* recorded from the Nematode worm, *Trilobus gracilis*.

A diversion is necessary here. The parasite, *Herpetomonas* (*Leptomonas*) *bütschlii*, briefly described by Bütschli in 1878 and named by Saville Kent in 1880, emphatically needs re-investigation. According to Bütschli, the organism was spindle-shaped or almost rod-shaped, bearing a long flagellum and possessing a contractile vacuole—a feature not present in flagellates latterly known as 'leptomonads.' No nucleus was observed by him. A darker, refractile granular mass behind the contractile vacuole may have been the blepharoplast, or even the nucleus. Saville Kent appears to have considered it to be the nucleus, and he incorrectly reproduced it as a vesicle in his 'Manual of the Infusoria.' The developmental cycle was unknown to Bütschli, and still remains so. The organism has not been studied by modern methods of technique, probably because of the difficulty of obtaining the host. It seems rather remarkable that a parasite which is somewhat shrouded in mystery should be made the type species of a genus, *Leptomonas*, which some workers would like to see supersede the well-known genus *Herpetomonas*. Moreover, Bütschli in 1884, in his account of the Mastigophora in Bronn's 'Tierreich,' considered the two generic names to be synonymous and retained the name *Herpetomonas*. The account of *H. muscae domesticae* by Prowazek having been shown by many competent investigators to be inaccurate—the so-called bi-flagellate characteristic being due to division—there is no need for the multiplication of genera and controversy on the same.

To return to the natural occurrence of herpetomonads in vertebrates. That these flagellates may occur naturally in mice is undoubted, as they have been seen and described by other workers in addition to Dutton and Todd. In May, 1909, while working in the Quick Laboratory, Cambridge, a *Herpetomonas* was found in life in the fresh peripheral blood of a mouse. It was seen almost simultaneously and independently by Prof. Nuttall, Dr. Porter and myself, but the infection was slight. The parasite has been seen



since, but again the infection was scanty. These observations have just been published by Fantham and Porter. The parasite is very probably *Herpetomonas pattoni* (Swingle), first seen in rat-fleas, as some of these ectoparasites were breeding in the rat cages in the animal house in which the mice were kept.

The so-called herpetomonad stages of trypanosomes are probably natural herpetomonads of vertebrates co-existing with trypanosome infections. This subject is discussed on page 345.

## V. THE OCCURRENCE OF HERPETOMONADS IN PLANTS

In 1909 Lafont announced the discovery in Mauritius of a *Herpetomonas*—or *Leptomonas*, as he called it—in the latex of a species of *Euphorbia* or spurge. Confirmation followed rapidly, and the flagellate (*H. davidi*) is now known in various species of *Euphorbia* in neighbouring islands, in India, Africa, Portugal, and other places (see França, 1914). In some of these places Hemiptera were found crawling over the plants, and herpetomonads were also found in the gut of the Hemiptera. Some of the parasitised plants were diseased, and the malady has been termed 'flagellosis.'

The flagellate parasite of the spurge plants is probably an insect flagellate, coming from the Hemiptera infesting the plants. The herpetomonad has probably secondarily invaded the plant tissue, having adapted itself so that it is now able to live in the latex of the plants. Encysted or post-flagellate stages of *H. davidi* have been obtained in cultures.

Nearly three years ago I was informed, by a competent authority, that a number of *Euphorbia* containing herpetomonads grew outside a certain hospital situated in an area in which kala-azar was endemic, and in which kala-azar patients were being treated. The shrubs were infested by insects. It seems remarkable that no attempt was made to trace a possible connection between the plant herpetomonad and kala-azar, doubtless such a possibility was considered too remote. Remarks of mine regarding a possible connection were received by my informant with polite incredulity, which is not surprising, since the wisdom of lecturing on *Herpetomonas* and *Crithidia* to students of tropical medicine has been questioned more than once.

## VI. EXPERIMENTS WITH INSECT FLAGELLATES BELONGING TO THE GENERA *HERPETOMONAS* AND *CRITHIDIA*

Thanks to the remarkable series of experiments recently carried out by Laveran and Franchini in Paris, and by Fantham and Porter in England, we now know that a number of species of *Herpetomonas* and *Crithidia*, naturally occurring in insects, may be successfully inoculated into or fed to mammals, especially rats and mice. The flagellates live and multiply in the vertebrates and become pathogenic thereto, the symptoms chiefly resembling those of kala-azar. It must not be supposed, however, that success is attained in every experiment, though a large proportion are positive, especially if the vertebrate hosts are young. (Compare the prevalence of leishmaniasis in *young* people (children) in the Mediterranean region.)

In the insect host the *Herpetomonas* or *Crithidia* is a natural or specific parasite, and its effects on its host are not marked. The life-cycle of the organism in the invertebrate consists of a leishmaniform pre-flagellate stage, gradually growing into a flagellate form and followed by a resistant post-flagellate, leishmaniform stage adapted for extra-corporeal life and for transmission to a new host. When such a parasite finds its way, either by inoculation or by feeding, into a susceptible vertebrate, it can assume again leishmaniform or flagellate facies. The mode of infection of the vertebrate in nature seems to be contaminative, either by its food or through an already existing abrasion or puncture on the surface of its body. Cases in which the flagellate-infected insects have been allowed to suck the blood of vertebrates have proved negative. Further, Fantham and Porter have brought forward experimental evidence which shows that post-flagellate forms of the parasites are best adapted to begin life in a vertebrate host.

In certain cases leishmaniasis may be derived from reservoir animals, such as man and various other warm- and cold-blooded vertebrates (see page 344), in other cases they may arise *de novo*.

Laveran and Franchini have successfully introduced into mammals *Herpetomonas ctenocephali* from the gut of the dog-flea, *H. pattoni* from the gut of the rat-flea, *Crithidia fasciculata* from the gut of *Anopheles maculipennis*, and *C. melophagia* from the gut of the sheep-ked.

Experiments have also been made with *H. muscae domesticae*. The mammals used were chiefly rats and mice.

Fantham and Porter (1914) went further, and showed that flagellates occurring in insects unassociated with the experimental vertebrate may be introduced therein. Their conclusions were as follows :—

- '1. Insect flagellates, e.g., *Herpetomonas jactatum* (Léger) from *Nepa cinerea*, and *Herpetomonas ctenocephali* (Fantham), parasitic in the dog-flea, *Ctenocephalus canis*, can live inside certain vertebrates (e.g., mouse and dog, respectively) and can multiply therein. This we have shown experimentally.
- '2. If such flagellates be inoculated intraperitoneally, or are fed by the mouth in food, the flagellates can find their way into the blood stream and internal organs (e.g., liver, spleen, bone-marrow) of the vertebrate host.
- '3. The insect flagellates are pathogenic to the vertebrates experimented upon, producing symptoms like those of leishmaniasis (kala-azar).
- '4. The oval post-flagellate forms appear to be more capable of developing in vertebrate hosts than are other stages of the herpetomonad parasite of the insect.
- '5. It may be expected that the various leishmaniasis, occurring in different parts of the world, will prove to be insect-borne herpetomoniasis.' (According to some, leptomoniasis would probably be considered correct, but see page 340.)

In a later paper further experiments were recorded by Fantham and Porter, and the researches were also extended to cold-blooded vertebrates. The conclusions were :—

- '1. Herpetomoniasis can be induced in various warm- and cold-blooded vertebrates when the latter are inoculated or fed with herpetomonads occurring in the digestive tracts of various insects. The infection produced and the protozoal parasites found in the vertebrates resemble those of human and canine leishmaniasis.
- '2. An infection can also be induced in certain vertebrates when they are fed or inoculated with *Crithidia geridis*, and both flagellate and non-flagellate stages occur therein, but no transition to a trypanosome was found.

- '3. The following Flagellata have proved pathogenic to warm-blooded mammals when the latter have been fed, or inoculated subcutaneously or intraperitoneally with them—*Herpetomonas jaculum*, *H. stratiomyiae*, *H. pediculi* and *Crithidia gerridis*. The hosts used were mice of various ages. That *H. ctenocephali* can infect dogs has already been shown by us.
- '4. *Herpetomonas jaculum* and *Crithidia gerridis* have also been successfully fed or inoculated into cold-blooded hosts, namely, fishes (*Gasterosteus aculeatus*), frogs, toads, lizards (*Lacerta vivipara*) and grass snakes (*Tropidonotus natrix*).
- '5. As we have previously stated, we believe that leishmaniasis are arthropod-borne herpetomoniasis, and that these maladies have been evolved from flagellates of invertebrates (especially herpetomonads of insects) which have been able to adapt themselves to life in vertebrates.
- '6. In areas where leishmaniasis are endemic, an examination should be made of all insects and other invertebrates likely to come into contact with men or dogs or rats and mice, in order to ascertain if these invertebrates harbour herpetomonads. Preventive measures should be directed against such invertebrates, especially arthropods. Further, it is likely that certain vertebrates, such as reptiles and amphibia (especially those that are insectivorous), may serve as reservoirs for leishmaniasis or, as they should preferably be termed, herpetomoniasis. From such reservoirs the herpetomonads may reach man by the agency of ectoparasites or flies, especially such as are sanguivorous.'

## VII. SOME INFERENCES

The immediate and important inference to be drawn from the experiments recorded in the previous section is that in them we see 'leishmaniasis in the making,' as was pointed out by Fantham and Porter in December, 1913, in these *Annals*. Also canine leishmaniasis is probably herpetomoniasis due to *H. ctenocephali* in dogs.

A further imperative inference is that probably only one species

of *Herpetomonas* is concerned in adapting itself to life in vertebrates in different parts of the world. This species is known under various names, such as *H. pattoni*, *H. ctenocephali*, *H. pediculi*, and also as *H. donovani*, *H. infantum*, *H. tropica*. These are probably merely physiological races of a herpetomonad which is very like *H. jaculum*, briefly described by Léger in 1902 from the gut of the Hemipteran, *Nepa cinerea*. This herpetomonad under different conditions of environment produces pathogenic effects in very varying degrees in different vertebrates, from zero as in Dutton and Todd's mice to high mortality as in Indian kala-azar, and probably zero again in cold-blooded hosts. It is also a flagellate which can probably live in invertebrates not already recorded as being infected.

At various times statements have been made that herpetomonad stages of trypanosomes occur in cultures. The invertebrate life-cycles of all the trypanosomes, which so far have been well investigated, lend no support to the presence of a herpetomonad stage therein. It is highly probable that the so-called herpetomonad stages of trypanosomes were really cultures of scanty herpetomonad infections co-existing with trypanosome infections. It is known that such invertebrates as rat-fleas may be infected with both herpetomonads and trypanosomes at the same time. The different discussions in the past, often acrimonious, as to what form of flagellate occurring in an invertebrate constituted part of the life-cycle of a trypanosome or otherwise, are thus to be deplored.

Before concluding, attention may be drawn to some remarkable and far-seeing suggestions regarding kala-azar published by Rogers in 1905. He wrote that 'the stomach of some blood-sucking insect is the most likely place in which to find the natural development of the extra-corporeal stage of the parasite, and that some such insect is the most likely carrier of the infection. . . . But as to the most likely kind of insects to carry the infection we are on more uncertain ground, for experiment alone can determine this. Nevertheless, it is worth while to discuss which are the most probable kinds in order that precautions may be taken against them without waiting for absolute proof to be obtained.' Especial notice is drawn to the last sentence, published ten years ago, but not yet acted upon fully from the point of view of preventive measures. However, exception must be made for the excellent researches and preventive measures undertaken by

Dodds Price, at the suggestion of and latterly in collaboration with Rogers, in the Assam tea gardens. He has reduced the mortality due to kala-azar enormously by segregating the infected, by moving coolie lines about 300 yards from older infected ones, and by having new coolie lines placed on clean sites.

It has remained for workers in laboratories in temperate zones, away from leishmaniasis material, again to point the way to preventive measures and to indicate the origin of the disease.

### VIII. SUMMARY

The significance of the herpetomonad stage of *Leishmania*, of the recent announcements that such stages occur in man, and of the presence of natural herpetomonads in other vertebrates (for example, mice), are discussed. It also recalled that insect herpetomonads can invade and live in plant-tissues.

The experiments on the introduction into different vertebrates of various species of *Herpetomonas* and *Crithidia* parasitic in insects by Laveran and Franchini, using mammals, and by Fantham and Porter, using both warm- and cold-blooded vertebrates, are summarised and discussed.

It is inferred that the various leishmaniases are due to a herpetomonad of invertebrates which, under different conditions of environment, produces pathogenic effects in very varying degrees in different vertebrates, from zero, as in the mice described by Dutton and Todd in 1903, to high mortality as in Indian kala-azar, and probably zero again in cold-blooded hosts. It is also a flagellate which can probably live in invertebrates not already recorded as being infected. A human reservoir of leishmaniasis may occur in some places, while warm- and cold-blooded vertebrates may also function as the same.

It is highly probable that the so-called cultural herpetomonad stages of trypanosomes were really cultures of scanty herpetomonad infections co-existing with trypanosome infections.

It is recalled that Rogers in 1905 published that 'it is worth while to discuss which are the most probable kinds [of insect transmitters of kala-azar] in order that precautions may be taken against them without waiting for absolute proof to be obtained.' Although these remarks were published ten years ago, little has been done directly in the way of the preventive measures suggested. A notable exception, however, is the work of Dodds Price in Assam.

ADDENDUM.—/June 28, 1915.

Since writing this paper my attention has been drawn to a recent note by Archibald, who writes: 'Epidemiological and experimental evidence does not support the theory that kala-azar in the Sudan is transmitted by a biting insect. A more probable source of infection appears to be some intermediate host, whose habitat is in water.' (*Rept. of Advisory Committee for Trop. Dis. Resch. Fund* for 1914, p. 116—published in April, 1915.) In this connection, see my remarks on the contaminative method of infection in paragraph 2 on page 342.

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# NOTES ON THE BIONOMICS OF *GLOSSINA PALPALIS* IN SIERRA LEONE, WITH SPECIAL REFERENCE TO ITS PUPAL HABITATS

[*Being the First Report of the Thirty-second Expedition of the  
Liverpool School of Tropical Medicine, 1914-1915*]

BY  
WARRINGTON YORKE  
AND  
B. BLACKLOCK

(*Received for publication 21 April, 1915*)

PLATES XXVII-XXXIII AND MAP

## INTRODUCTION

The Thirty-second Expedition of the Liverpool School of Tropical Medicine was dispatched to Sierra Leone on November 18th, 1914, and returned to England on April 2nd, 1915.

The primary object of this expedition was to find a site suitable for the permanent research laboratory which the School proposes to erect in Sierra Leone. Thanks to the kindness of the Administration, we were enabled to approach the subject under the most favourable circumstances and to obtain information of the local conditions as regards population and incidence of disease. After other factors, such as healthiness, cost of erection and maintenance of the laboratory, and accessibility from England, had been considered, we were in a position to make definite recommendations which were embodied in a report to the Committee of the School.

During our sojourn in the country we made a survey of the chief parasitic diseases of man and domestic stock, with a view to ascertaining the lines along which research could with most advantage be directed in the laboratory which it is hoped to establish.

The scientific research undertaken by us deals chiefly with the bionomics of *Glossina palpalis*. The results of this and other work accomplished will be published in a series of papers in these Annals.

We desire to express our deep indebtedness to His Excellency

the Governor, Sir Edward Merewether, to the Principal Medical Officer, Dr. T. E. Rice, and to the Senior Sanitary Officer, Dr. R. H. Kennan, for the invariable kindness and courtesy shown to us during our visit to the Colony, and for the assistance rendered on every possible occasion.

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Some four or five miles from Freetown, at the mouth of the Sierra Leone River, lies the Cape Lighthouse Peninsula. This triangular piece of land, of which the long axis is approximately north and south, is separated from the mainland by Aberdeen Creek. On the other two sides the Peninsula is bounded by the sea. It is roughly three miles long, by one and a half miles broad at its base, with an area of about threequarters of a square mile. It tapers rapidly and runs as a narrow strip about 100 yards wide for nearly two miles, until it joins the mainland at its southern extremity. The Creek is fringed by a dense growth of mangroves, which in certain places is as much as half a mile broad. On the northern side the shore is rocky, and on the western side there is a gently sloping beach of fine white sand (see map).

While oil palms (*Eloeis guineensis*) are found all over the Peninsula, in certain parts they occur exclusively, the appearance of dense undergrowth being due entirely to the presence of innumerable young palms (Pl. XXVII). In such places which are found especially in the region adjacent to the mangroves fringing Aberdeen Creek, and in that portion of the Peninsula on which the lighthouse and isolation camp are situated, there is deep shade, and little or no long grass. Between Aberdeen and the isolation camp the growth of palms is not so dense, and consequently the shade is less; in this locality other trees are found, and there is a considerable amount of long grass. Between the isolation camp and Man of War Bay is a small area in which oil palms, although present in considerable numbers, do not predominate to the same extent as elsewhere, many other trees being present together with a dense undergrowth of shrub. Besides oil palms, cocoanut palms, mangoes and cotton trees are represented; there are also a few baobabs, and in the village of Aberdeen bread-fruit trees, guavas, plantains and golden plum trees.



Dense growth of young oil palms.



\_\_\_\_\_



Hollow baobab constituting a breeding ground of *Glossina palpalis*.



The population of the Peninsula is fairly large; in the town of Aberdeen are some 500 inhabitants, whilst at the Lighthouse and isolation camp are 50 more, and in addition there are a few natives living in isolated dwellings. Where the Peninsula joins the mainland is the native town of Lumley, with over 400 inhabitants.

Goats, sheep, dogs, domestic fowls and a solitary cow were found, apparently in good health. Regarding the wild fauna of the Peninsula nothing very definite is known; monkeys (*Cercopithecus* sp.), squirrels, bats, rats and mice are numerous, as are also many varieties of birds, including bush fowl, guinea-fowl, ducks and sea birds. A few antelope, bush buck and duiker, were seen, but they are scarce.

The only fresh water on the Peninsula, apart from the wells themselves, is the practically insignificant overflow from two or three springs.

At the beginning of the dry season—December and January—the tsetse-fly *Glossina palpalis* was found in fair numbers all over the Peninsula. A similar prevalence of this fly is recorded by Dr. W. A. Young, who had spent a month in the locality during the previous rainy season.

Owing to the circumscribed nature of the Peninsula, its relatively small size, its proximity to Freetown, and to the fact that cases of sleeping sickness have been found in the vicinity, the place seemed well adapted for studying the bionomics of *Glossina palpalis*. We were compelled to confine ourselves to one or two of the more important aspects of the question, as the time at our disposal was limited.

Although *Glossina palpalis* is known to be widely distributed over the West Coast of Africa, and in many places the distribution of the fly has been carefully mapped out, but little is known of its pupal habitats in this region. We decided, therefore, to commence our investigations by a thorough search for pupae. Curiously enough our earliest effort in this direction was successful, as in the first place examined no less than eighteen empty pupal cases were discovered. The site (Pl. XXVIII) was a large cavity in the trunk of a baobab tree. The cavity was conical in shape, having the ground as its base; the apex of the cone was some 12 to 14 feet above the ground. The entrance to this chamber was a large triangular

opening, about 4 feet wide at the base, which was on a level with the ground, and about 7 feet high at its apex; it faced south-west. Owing to the proximity of oil palms and a large mango, no direct sunlight entered the cavity through this aperture after about 8.30 a.m. One or two small slit-like apertures, about 3 or 4 feet from the ground, admitted a little light to the chamber from its northern aspect. The floor of the cavity was, as already mentioned, flush with the ground, and was composed of a fine dry laterite gravel covered with dead leaves and débris from the inner surface of the tree trunk, which was ragged and rugose. The pupal cases were found after removal of the dead leaves, lying either superficially or within half an inch of the surface of the ground. Pupal cases were not found in the numerous crevices of the tree trunk itself, many of which contained earth probably carried there by ants.

This breeding place was about six yards from the main path leading to the lighthouse, and about 200 yards from the latter. It was at least 100 yards from the sea, and not less than a quarter of a mile from the nearest fresh water, which consisted of a small well. It is interesting, and probably important, to note that pupal cases were only found within a foot of the trunk of the tree, and not in the centre of the floor of the cavity; the explanation of this peculiar distribution will be discussed later.

Many other places were then searched, but with little success. These included the intervals between the buttresses at the roots of cotton trees, dark crevices in rocks, and rock surface densely shaded by trees, the ground immediately under and overshadowed by fallen trees and boulders, the cavities at the top of stumps of trees which had been felled, the sand along the seashore and many other places of a similar nature. A solitary empty pupal case was discovered on the sand (Pl. XXIX, fig. 1) at the base of a palm tree growing on the margin of the sandy shore, and another was discovered lying on the ground in deep shade at the base of a similar tree situated in dense bush.

We then decided to make a systematic search for pupae on and around oil palms. With this object we chose at random a young palm (Pl. XXXI); the tree selected was within 15 yards of the road and about 200 yards of the lighthouse. It was surrounded and overshadowed by other oil palms, which in this region formed about



FIG. 1. Oil palms fringing sandy sea-shore.



FIG. 2. General view of palm country.







Young oil palm ; a few of the lowest petioles have been cleared from the right side.

95 per cent. of all the trees present. The trunk of an oil palm which has its lower petioles still unremoved, as is the case with most of the palms in the Peninsula, is by no means easy of approach. An impenetrable barrier is formed by the lower petioles which project out horizontally in all directions to a distance of 3 feet or more from the trunk. Frequently the tips of the lowest petioles dip into the ground, an arrangement which in conjunction with the fact that the petioles are armed with strong sharp spines effectually prevents any close examination of the ground in the immediate vicinity of the trunk of the tree (Pl. XXX and XXXI). Having first searched the ground beyond the range of the petioles and as far as possible under them without success, the petioles one by one were cut off at their place of origin from the trunk. The ground under each was then carefully searched. After removal of the dead leaves and light débris twenty empty pupal cases were discovered. The cases were found all round the trunk, lying, as a rule, not more than 12 inches from it; in every instance they were on the surface. The ground was dry and sandy, and covered with a certain amount of fine laterite gravel. The shade was dense, being derived not only from the petioles of the palm itself, but also from the close proximity of a large number of other palms, which almost completely cut off direct sunlight during the entire day.

The examination of the soil around the trunks of the trees was greatly facilitated by the use of a spade and newspaper. A shovelful of superficial soil was placed on the newspaper and spread out in a thin layer, when the puparia could readily be seen. By this means the soil at different depths was more accurately and expeditiously examined. The superficial layer to the depth of about half an inch was first removed and examined, and subsequently the deeper layers. It was thus shown that by far the majority of puparia were either actually on the surface of the ground or less than half an inch below the surface; the deeper layers contained very few pupal cases. The following observation illustrates this point. A palm having been stripped of all its lower petioles, the ground around it was cleared of dead leaves and twigs. The soil to an extent of about one yard from the trunk was then removed in layers and carefully examined for puparia. The

following was the result :—The most superficial half-inch contained fifty-one puparia. The soil between one-half inch and three inches in depth contained ten, that between three inches and six inches only two; whilst below this depth no puparia were discovered.

Adopting this procedure, we had not much difficulty in finding large numbers of puparia of *Glossina palpalis* in similar sites. Frequently between ten and twenty pupal cases were taken under a single palm, and on one or two occasions between twenty and thirty, whilst in two instances seventy-three and seventy-five puparia respectively were collected.

As a result of many observations of this kind, we are enabled to state, as regards this Peninsula, that under any oil palm which has not had its lower petioles removed, which stands in dense shade and the ground under which is dry and not too stony, a search for the puparia of *Glossina palpalis* would in all probability be successful. We do not mean to imply by this that, other things being equal, pupae do not exist where the ground is stony, but simply that they are more difficult to recognise on such a surface. We put this postulate to the test by examining a single spadeful of superficial earth removed from under the petioles close to the trunk of each of twenty oil palms in a region where practically every tree was a palm. In two instances only did we fail to find the puparia of *Glossina palpalis* by this means.

The angles which the petioles form with the trunk of the palm contain considerable quantities of earth and débris. These forks appear to be suitable for the deposition of larvae, and accordingly a very careful search for puparia was made. Before examining the contents of the angles, all the pupae lying on the ground to a depth of three inches were first removed with the aid of a spade, care being taken to disturb the petioles as little as possible. The ground around the trunk was then covered with newspapers and the petioles stripped from the tree, and all the soil and débris in the angles collected and examined. In this manner several palms were entirely stripped of petioles, whilst odd petioles were removed from many other palms (Pl. XXXI and XXXII). The result was striking, a solitary empty pupal case only being discovered in this situation.

At the base of other trees, especially cotton trees and mangoes, are what appear ideal pupal habitats from the point of view of shade and soil. The result of searching these places was, however,





Oil palms ; the lower petioles have been stripped from the tree on the left.





Young oil palm from which all the petioles on the right side had been stripped.





1



**PAGE NOT  
AVAILABLE**



FIG. 1. Mangrove swamp at high tide.



FIG. 2. Mangrove swamp at low tide.



decidedly disappointing, only an occasional puparium being discovered.

As might be expected, most of the puparia taken were empty. Out of a total of about 450 only twenty were unhatched.\* From a number of these *Glossina palpalis* emerged after they had been kept in the laboratory some days.

We have already referred to the fact that the Aberdeen Creek side of the Peninsula is fringed with extensive mangrove swamp. It is popularly supposed that there is some connection between the presence of these swamps and the occurrence of *Glossina palpalis*. In fact, the local name for the 'fly' amongst the natives is 'mangrove fly.' That portion of the swamp in the vicinity of Aberdeen village was carefully examined by us firstly for *Glossina palpalis*, and secondly for its pupae. It should be mentioned that while the roots of the mangroves are covered at high tide to a depth varying from a few inches to three or four feet, at low tide the sea recedes to a considerable distance beyond their outer fringe, leaving amongst them small collections of water and tiny streams, so that the mangroves are seen to be growing in soft sandy mud and pools of water (Pl. XXXIII). An inspection of the mangroves was made on several occasions, both at low and high water. The outer edge was examined on foot at low water, but a boat was necessary at high tide.

*Glossina* was found not only in the midst of the mangroves, but also at their outermost fringe, both at low and high water; this is of considerable interest, as at certain points where the tsetse was seen the distance from the dry land was at least half a mile. No trees were found growing amongst the mangroves below high water, although just above this point a few mangroves were noticed intermingled with the ordinary bush growth. The mangroves themselves afforded fairly good shade, and this, together with the fact that a considerable number of paths were cut through them to the fishing grounds beyond, and that the mangroves are inhabited by large numbers of small birds, mud fish, crabs, shellfish, snails, wading birds, etc., which may afford a supply of food for the tsetse, is probably sufficient reason for its presence.

The next question to be considered is, do the mangroves

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\* The extreme length and breadth of 16 unhatched pupae were measured. The following is a summary of the results:—Length: max. 6.1 mm., min. 5.6 mm; average 5.93 mm. Breadth: max. 3.6 mm; min. 3.0 mm; average 3.25 mm.

constitute a breeding ground for *Glossina palpalis*? Bagshawe (1911) writes: 'It is probably lost labour to look in moist soil and mud for pupae of tsetse flies; they have never been found in any situation which is not at least moderately dry.' Nevertheless, in view of the fact that the fly was found amongst the mangroves, we determined to investigate the point carefully. At low tide, with the aid of six assistants, all of whom had had considerable experience in finding pupae, we made an exhaustive examination of the sandy mud amongst the mangroves and also of the mangroves themselves. The latter, however, afford very few possible places for the lodgment of pupae, as the stems are smooth and narrow and do not present many forks or clefts in which pupae could lodge. Similar examinations were made at high tide over the same area. No puparia were found on the mud or floating on the water amongst the mangroves, nor were any discovered on the stems and branches.

As a result of this work, it appears clear that the tsetse which we observed in the mangrove swamps were not breeding there, but had travelled from the land. Support to this conclusion was furnished by examination of the bush immediately above high-water mark. Puparia were found there under oil palms in large numbers, and a few under other trees. Apart from the failure to discover puparia in the mangrove swamps such places do not appear to be suitable breeding grounds for tsetse-fly, as pupae which are deposited on sea-water or on ground which is covered by sea-water for a certain number of hours each day do not hatch, as is shown by the following experiment:—

EXPERIMENT.—Eighteen unhatched pupae found at the base of oil palms were placed in glass jars containing a small quantity of sand. Nine of the jars were half filled with sea-water for a period of three hours daily during a month. The pupae were observed to float horizontally on the surface of the water, the tubercles and stigmata being as a rule just below the surface. When the water was decanted at the end of the three hours, the pupae were left lying on the moist sand. This experiment was an attempt to imitate the conditions to which pupae deposited in mangrove swamp would be subjected, except that in the experiment they were exposed to water once a day only, whereas in nature the tides cover the mangrove swamps twice daily. At the end of a month the daily addition of water was discontinued and the pupae were left lying in the sand which soon became dry. No water was added to the other nine jars, the pupae in which served as controls. Six of the nine control pupae hatched within the time during which the experiment was continued, viz.: 35-60 days, depending on the date when the pupae were discovered. The flies emerged on the 1st, 10th, 20th, 23rd, 27th and 32nd days respectively after the commencement of the experiment. No flies emerged from the pupae which had been exposed to sea-water. The temperature was that of the laboratory 80°-86° F.

Carpenter's (1912) work on this subject is of great interest; he writes 'According to my experience pupae are only found in the driest possible situation, though always close to the water's edge; so that it would appear that contact with water is inimical to them. It will be seen, however, that they have a considerable capacity to resist the effects of submersion; moreover, they do not become mouldy (so long as they are alive) when kept in an atmosphere with the maximum degree of humidity. Their powers of resistance to sun are less in proportion.' He concludes from experiments that should any breeding ground be flooded at intervals by heavy rains the conditions would not completely destroy all the pupae. Even four successive submersions for twenty-four hours destroyed only half the number. Further experiments showed that it is rather the frequency of submersion than the total time that is adverse to the pupae. He states that a certain proportion can survive flotation for eight days, but none for ten days.

A general survey of the pupal habitats discovered by us in the Cape Lighthouse Peninsula reveals several important facts.

Firstly, fly and pupae are distributed more or less evenly over the whole extent of the Peninsula. They are certainly not limited to the immediate vicinity of water, both being found at least a quarter of a mile from the seashore. This is the more remarkable in view of the almost complete absence of fresh water. There is, so far as we are aware, no record in the literature of *palpalis* pupae being found in any situation except on the water's edge. Bagshawe (1908), who was the first to discover the pupae of *Glossina palpalis* in nature, writes 'One may for the present say that the larvae are dropped in shade, it may be of shrubs, it may be of bananas, within forty-five yards of water.' Fraser and Marshall (1909) state that the distance from high-water mark at which deposits were found was never more than fifteen yards, the usual distance being five yards; whilst Carpenter writes 'According to my experience pupae are only found in the driest possible situations though always close to the water's edge.' It must be noted, however, that these statements refer to East Africa. The observers quoted worked on Lake Victoria Nyanza, where conditions appear to be rather different from those obtaining in Sierra Leone. Whilst enormous numbers of flies are to be found in the former place, and they appear to be strictly

limited in their distribution to the lake shore and larger rivers, as regards the Cape Lighthouse Peninsula this is certainly not the case. *Glossina palpalis* is not nearly so numerous, nor is it limited to the immediate vicinity of water.

Secondly, pupae were concentrated on the earth close to the trunks of trees, and were not found to any extent on the trees themselves or on the ground between them. By far the majority of the puparia taken by us were less than one foot from a tree trunk. The explanation of this appears to be that the tsetse deposits its larvae in the most secluded and shady spot available. Possibly the larva is deposited whilst the fly is resting on the trunk or on the under surface of a lower petiole. Such a hypothesis would account for the peculiar distribution of the puparia in the hollow baobab referred to previously. Tsetses resting on the inner surface of the hollow trunk would drop their larvae on the ground close to the tree trunk, thus explaining the peripheral distribution of the puparia on the floor of the chamber. Similarly in the case of the oil palm, flies resting on the under surface of the lower petioles or on the tree trunk would also drop their larvae on the ground near the trunk.

The facts discovered by Moiser (1912) are of interest in this connection. He put men in trees at a height varying from 10 to 25 feet for an hour; not one of them reported having seen a fly, though several (*Glossina tachinoides*) were seen on the ground during the period. In order to determine where flies usually rest, he put up a large mosquito net (8 x 8 x 8 ft.) in the bush, thus enclosing a portion of the natural haunt of the fly. Eleven *Glossina tachinoides* were then liberated within the net. A number of them quickly disappeared from view, and after a few minutes' search he discovered them resting in an inverted position on the under side of small branches and twigs close to the ground. Branches between 6 inches and 2 feet from the ground were those most frequently occupied by the tsetses, and in order to observe them closely Moiser had to lie on the ground. Subsequently flies were found at rest on the under surface of small branches outside the net.

Thirdly, the ground at the base of oil palms appears to be a most suitable breeding place for *Glossina palpalis*. In this region puparia of *Glossina palpalis* can be found under almost any oil palm which has not had its lower petioles removed, which stands in dense

shade, and the ground under which is dry and not too stony. Moreover, *Glossina palpalis* is able to breed in a locality where oil palms of various sizes and ages afford the sole shelter. The lower surface of a petiole is broad and smooth and well protected from the sun, and tsetse have been observed in this situation. A fly in this position is hidden from view except to anything actually on the ground, and is also to an extent protected from molestation by the sharp strong spines with which the petioles are armed. In so far as the Cape Lighthouse Peninsula is concerned, the situations in which pupae are deposited are hidden from view, probably as a protective measure for the fly undergoing parturition and for the newly deposited larva against birds, a view which would explain the absence of puparia in many spots, such as the spaces between the buttresses of the trunks of cotton trees, otherwise apparently well adapted as pupal habitats.

Zupitza (1908), working at Duala in the Cameroons, recorded that he had found the puparia of *Glossina palpalis* in the humus and moss in the forks of branches and in the cracks in the bark of all trees, especially in the angles of leaf sheaths of palms at a height of a few centimetres to three and a half metres above the ground. He did not look for them at a higher level. They were never found in the dry mould of hollow trees, nor in or on the ground. The observations of Zupitza are in curious contradistinction to those made by us. The first pupal habitat discovered by us was the ground forming the floor of the chamber in the baobab trunk. Moreover, whilst we constantly found puparia on the ground close to the trunk of oil palms, only a single empty case was discovered in the angles between the petioles and the tree trunk. As already mentioned, we most carefully investigated this place as a possible pupal habitat, entirely stripping the petioles from three or four palms on the ground under which large numbers of pupae had been found, and examining the débris between them and the tree trunks without finding any pupae. In addition, odd petioles were stripped from many other palms with equally negative results.

The explanation of this apparent discrepancy is cryptic. We are not able to suggest any reason why *Glossina palpalis* should in one locality deposit its pupae on the ground and in another on the tree itself, unless in the latter case the ground happened, through

moisture or some other unknown condition, to be an unsuitable pupal habitat. Zupitza states that in the angles between the petioles and the trunks of the palms the pupae would be kept moist, but that they would be in no danger of being drowned or washed away by rain-water running down the trunk. He is evidently under the impression that some amount of moisture is necessary, as he never found them in the dry moss in the holes of trees, nor in or on the ground. This is not our experience, as they were almost invariably found lying on or just under the surface of perfectly dry ground.

Before leaving this subject, it is of interest to consider what steps it would be necessary to take in order to clear the Peninsula of tsetse. In view of the fact that the fly is ubiquitous in the area under consideration and that its pupae have a like distribution, it is quite obvious that no localised or partial clearing could be recommended as likely to eradicate the fly. Moreover, in districts where practically all the vegetation consists of oil palms, wholesale removal of these cannot, for economic reasons, be contemplated. Yet we are faced with the fact that it is under the young palms that the tsetse-fly find their best breeding place; any method to be practicable must not interfere with the economic value of the trees. The point that has to be considered, therefore, is whether any measures short of actual destruction of the trees would suffice to get rid of the fly. In our experience the ground at the base of an oil palm which has had its trunk cleared of petioles does not constitute a breeding place of *palpalis*. The soil at the base of many such trees was searched without success. We are convinced that it is the presence of the lower petioles that renders young oil palms such excellent breeding places. Removal of these would, in our opinion, destroy the breeding grounds of *palpalis*, and by this means such localities could be freed of fly without loss by damage to the trees. Such a procedure would be laborious, but it must be remembered that the petioles when once removed do not grow again. In old palms the lower petioles are absent; as the tree grows the lower, that is the older, petioles become dry and rotten and are easily stripped off, but in very young palms they are much tougher, and hence more difficult to remove. Naturally, in those places where shrub growth, other than young oil palms, occurred, it would have to be removed.

It may not be considered advisable on economic or epidemiological grounds to attempt to rid the Peninsula of tsetse at the present time. If, however, this can be undertaken as an experiment, results of the utmost value would be obtained, and the information gained could be applied in districts where for economic or epidemiological reasons it might be of vital importance to exterminate *Glossina palpalis*. Owing to its proximity to Freetown, its small size and its well circumscribed character, the Cape Lighthouse Peninsula is eminently suitable for an experiment of this nature.

### CONCLUSIONS

1. The breeding grounds of *Glossina palpalis* are not so strictly limited to the immediate vicinity of water as has hitherto been thought; they may occur quite independently of fresh water and at least a quarter of a mile from sea water.
2. Although *Glossina palpalis* is to be found in considerable numbers in mangrove swamps and may travel in these to a distance of at least half a mile from dry land, the swamps do not constitute a breeding ground of the fly.
3. The pupae of *Glossina palpalis* do not hatch when subjected to daily flotation on sea water.
4. The ground around the trunk of oil palms (*Eloeis guineensis*) which have not been stripped of their lower petioles constitutes an excellent breeding place for *Glossina palpalis*.
5. *Glossina palpalis* can breed in localities in which practically the only tree is the oil palm.
6. Stripping the oil palm of the lower petioles would suffice to destroy the breeding ground in such localities.

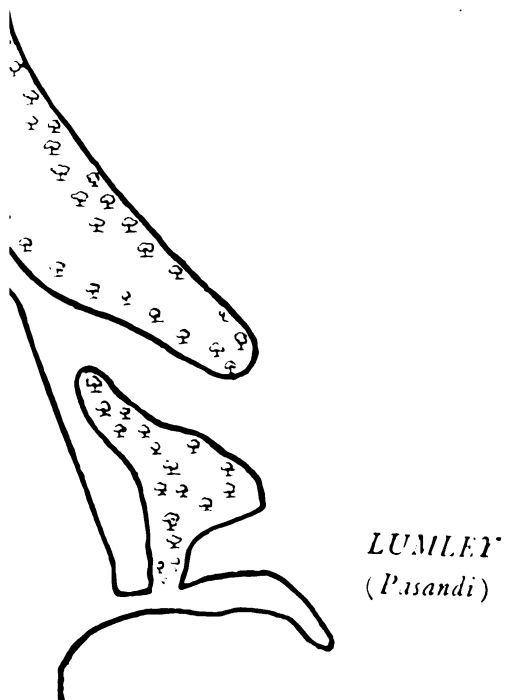
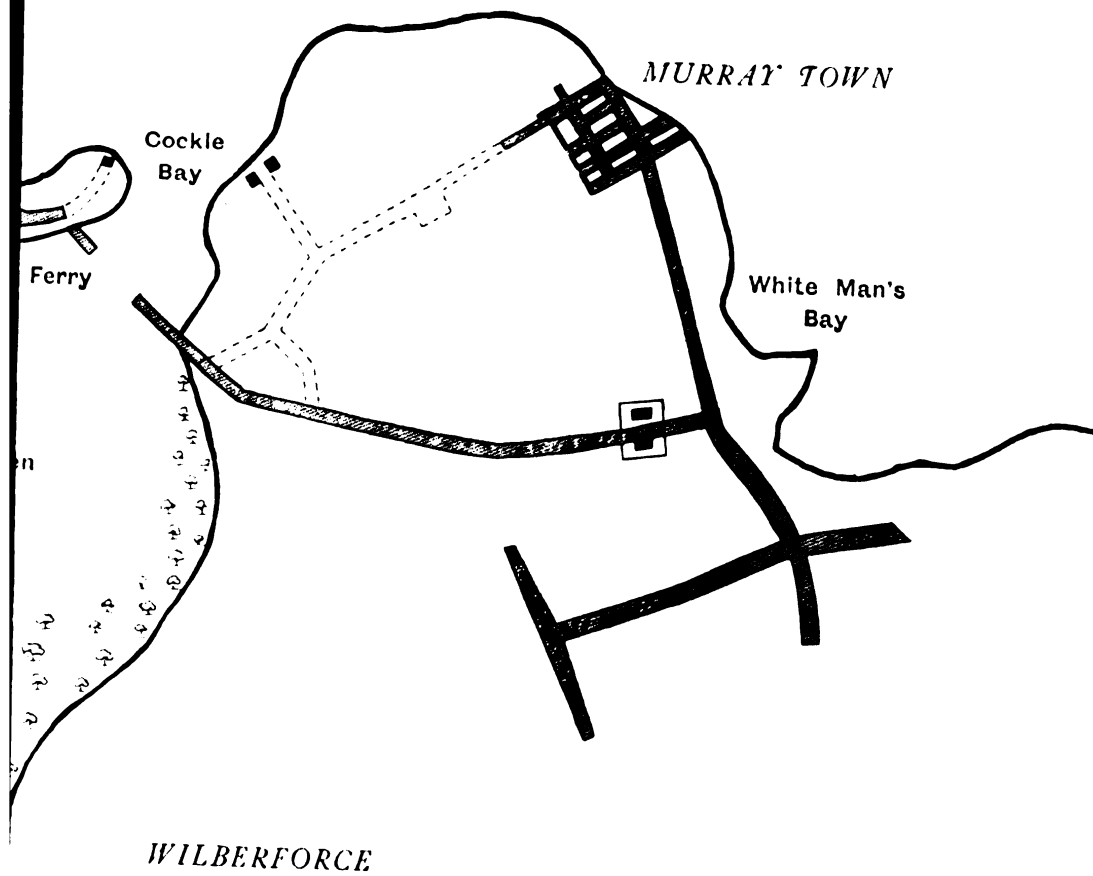
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# F CAPE PENINSULA

1" = 1 Mile





FOOD OF *GLOSSINA PALPALIS* IN  
THE CAPE LIGHTHOUSE PENINSULA,  
SIERRA LEONE

[*Being the Second Report of the Thirty-second Expedition of the  
Liverpool School of Tropical Medicine, 1914-1915.*]

BY

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AND

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Although there is a certain amount of evidence to show that *Glossina palpalis* can take up water and vegetable juices, practically all observations indicate that for its continued existence vertebrate blood is necessary. In view of the small amount of time at our disposal, and of the comparative scarcity of *Glossina palpalis*, we were able to examine only 200 flies in respect of the nature of the blood found in their intestinal tract. The tsetse were dissected immediately they were brought into the laboratory. As at the same time the flies were examined for the presence of trypanosomes, they were dissected in the manner described by Lloyd (1912). Briefly it consists in splitting the dorsum of the thorax longitudinally with a cataract knife, and then drawing out the salivary glands by gentle traction on the head. Usually the glands are removed intact attached to the head, the oesophagus breaking off at the point where it enters the pharynx; occasionally they break across when only partially withdrawn, but in this case are easily caught up with a fine pair of forceps. The proventriculus is then sought at the ventral surface of the thorax, and is drawn out with the sucking stomach and most of the intestine. The posterior portion of the hind-gut and rectum are removed by cutting off the last segment of the abdomen. The gut contents and those of the salivary glands and of the proboscis were examined between a slide and coverslip in a fresh unstained condition.

Recognisable red blood cells were seen in 16 of the 200 flies thus examined. It is necessary to point out here that only those flies in which definite red blood corpuscles were seen are recorded as containing blood. Practically every fly had in its mid-gut a certain quantity of pigmented material, the colour of which varied from bright red to brown or black. Such pigmented material does not, however, necessarily imply the presence of red blood corpuscles; on microscopical examination it was usually found to consist of granular debris and globules appearing red or brown in colour, and crystals which in many cases were probably haemin or haemoglobin crystals. Although it is highly probable that this pigmented material was derived from blood, still one could not be certain, and, therefore, in the following table only those flies in which definite red blood corpuscles were encountered are considered as containing blood.

TABLE I.—Result of examination of freshly-caught wild *Glossina palpalis* for recognisable blood corpuscles

	Number examined	Number in which recognisable mammalian red corpuscles were seen	Number in which recognisable nucleated red corpuscles were seen
Males ... ..	113	8	0
Females ... ..	87	6	2
Total ... ..	200	14	2

From the limited number of observations made, it appears that eight per cent. of freshly caught *Glossina palpalis* in this Peninsula contain recognisable red blood corpuscles—mammalian blood in seven per cent., and nucleated red cells of an undetermined nature in one per cent. The chief source of mammalian blood on the Peninsula is probably man and his domestic stock—goats, sheep, and dogs. In addition there are a few antelope—bushbuck and duiker were seen—and a considerable number of monkeys (*Cercopithecus* sp.) squirrels and bats, rats and mice. It is interesting to note that the mammalian red cells seen in the fourteen flies observed to contain these, were of the large type and readily distinguishable from the small variety found in sheep and goats.

The source of nucleated blood corpuscles is apparently much larger. Birds and lizards are numerous, and in the mangrove swamps there are vast numbers of mud-fish, crabs, shell-fish, snails, wading birds and sea birds. No crocodiles occur. In view of the fact that the reservoir of nucleated blood corpuscles is probably much greater than that of non-nucleated red cells, it is interesting to find mammalian blood in fourteen *Glossina palpalis* and nucleated red cells in only two, an observation which appears to indicate that *Glossina palpalis* either prefers mammalian blood or finds it more easy to obtain. The flies were captured at various parts of the Peninsula, but owing to its small size none of these localities was more than a quarter of a mile from human habitation—either the village of Aberdeen or the settlements at the isolation camp and the Cape Lighthouse.

In order to appreciate the real meaning of the results obtained by such an examination of the gut of freshly caught tsetse in respect of blood corpuscles, it is of importance to have at our disposal some data regarding the length of time red blood cells can be recognised in the intestine of a fly after feeding. To obtain this information freshly caught tsetse were fed on a rat or fowl and dissected after various intervals, and the gut contents examined for the presence of red blood cells. The results are given in the Tables II and III.

In the case of mammalian blood it is seen that in over 90 per cent. of the flies red cells can be recognised 24 hours after a feed, but in only 40 per cent. of those examined after 48 hours, whilst in no instance were definite red cells noted after 72 hours. The fowl red blood cells were found to be recognisable for longer periods; after 24 hours 100 per cent. of the flies showed nucleated red blood cells, whilst after 48 hours red blood cells were seen in 60 per cent., and after 72 hours in 40 per cent. The temperature was that of the laboratory, 80°-86° F. Possibly to a certain extent this difference is to be explained by the fact that the nucleated red blood cells of the fowl are more characteristic, and hence more easily recognised than are the non-nucleated red cells of mammals. Of course, these figures are approximate, as only 64 tsetse were employed in the experiment: they are, however, sufficiently accurate for practical purposes, although some observers have recorded, in the case of isolated flies, that blood could be found after much longer

intervals. Thus in the case of *Glossina morsitans*, Lloyd (1913) observed fowl red cells in a clot in the sucking stomach several weeks after the fly had last fed on a fowl, monkeys having been used as blood donors in the interval. Nevertheless, these figures show

TABLE II.—Experiment to ascertain the length of time rat red blood corpuscles can be recognised in *G. palpalis*

No.	Date fed	Date examined	No. of hours after feed	Sex	Result of examination. Recognisable blood cells present or absent	Percentage of flies in which blood was found
1	17.12.14	18.12.14	24	♂	+++	92
2	17.12.14	18.12.14	24	♂	+++	
3	17.12.14	18.12.14	24	♂	+++	
4	17.12.14	18.12.14	24	♀	+++	
5	17.12.14	18.12.14	24	♀	+++	
6	17.12.14	18.12.14	24	♂	+++	
7	17.12.14	18.12.14	24	♀	+++	
8	17.12.14	18.12.14	24	♂	++	
9	28.1.15	29.1.15	24	♂	—	
10	28.1.15	29.1.15	24	♂	+++	
11	2.2.15	3.2.15	24	♂	+++	
12	2.2.15	3.2.15	24	♂	+++	
13	28.1.15	30.1.15	48	♂	—	40
14	28.1.15	30.1.15	48	♀	—	
15	28.1.15	30.1.15	48	♀	+++	
16	28.1.15	30.1.15	48	♀	—	
17	28.1.15	30.1.15	48	♀	+++	
18	2.2.15	4.2.15	48	♀	—	
19	2.2.15	4.2.15	48	♂	—	
20	2.2.15	4.2.15	48	♀	+	
21	2.2.15	4.2.15	48	♂	—	
22	2.2.15	4.2.15	48	♂	+++	
23	28.1.15	31.1.15	72	♂	—	0
24	28.1.15	31.1.15	72	♂	—	
25	28.1.15	31.1.15	72	♀	—	
26	2.2.15	5.2.15	72	♀	—	
27	2.2.15	5.2.15	72	♂	—	
28	2.2.15	5.2.15	72	♂	—	
29	2.2.15	5.2.15	72	♀	—	
30	2.2.15	5.2.15	72	♀	—	
31	2.2.15	5.2.15	72	♂	—	
32	2.2.15	5.2.15	72	♀	—	
33	2.2.15	5.2.15	72	♂	—	
34	21.1.15	25.1.15	96	♂	—	

that as a general rule mammalian (rat) red blood cells are no longer recognisable after a period of 72 hours, whilst fowl red cells are observed in only 40 per cent. of cases after a similar period. This information is of importance in connection with that obtained by

examining the blood found in freshly caught tsetse-fly. When the statement is made that only seven per cent. of such flies contain mammalian blood, it must be borne in mind that if the flies had all fed on a mammal 48 hours previously, we would expect to find red cells in only 40 per cent., whilst if they had fed 72 hours previously very few would contain recognisable red cells.

TABLE III.—Experiment to ascertain the length of time fowl red blood corpuscles can be recognised in *G. palpalis*

No.	Date fed	Date examined	No. of hours after feed	Sex	Result of examination. Recognisable blood cells present or absent	Percentage of flies in which blood was found
1	28.1.15	29.1.15	24	♂	+++	100
2	30.1.15	31.1.15	24	♂	+++	
3	30.1.15	31.1.15	24	♂	+++	
4	30.1.15	31.1.15	24	♂	+++	
5	1.2.15	2.2.15	24	♀	+	
6	1.2.15	2.2.15	24	♀	+++	
7	1.2.15	2.2.15	24	♀	+++	
8	1.2.15	2.2.15	24	♀	+++	
9	1.2.15	2.2.15	24	♀	+	
10	1.2.15	2.2.15	24	♀	++	
11	28.1.15	30.1.15	48	♂	—	60
12	28.1.15	30.1.15	48	♂	—	
13	28.1.15	30.1.15	48	♂	—	
14	28.1.15	30.1.15	48	♂	+++	
15	28.1.15	30.1.15	48	♀	+++	
16	1.2.15	3.2.15	48	♂	+	
17	1.2.15	3.2.15	48	♂	+++	
18	1.2.15	3.2.15	48	♂	—	
19	1.2.15	3.2.15	48	♂	+++	
20	1.2.15	3.2.15	48	♂	+++	
21	28.1.15	31.1.15	72	♂	—	40
22	28.1.15	31.1.15	72	♂	+	
23	28.1.15	31.1.15	72	♀	+	
24	28.1.15	31.1.15	72	♂	—	
25	28.1.15	31.1.15	72	♀	—	
26	30.1.15	2.2.15	72	♂	—	
27	30.1.15	2.2.15	72	♀	+++	
28	30.1.15	2.2.15	72	♀	—	
29	30.1.15	2.2.15	72	♂	++	
30	30.1.15	2.2.15	72	♂	—	

The question whether tsetse-fly take up other food than blood is one which is difficult to decide. Stuhlmann (1907) and Degen (1909) came to the conclusion that they did not, but Maugham (1911) states that he has seen tsetse-flies sucking vegetable juices on two occasions. In 1905 he observed a *Glossina morsitans* alight on a

stem of young marsh grass (*Phragmites communis*) and deliberately insert its proboscis and unmistakably suck for a period of about three and a half minutes. At this stage Maugham caught the fly, and found on examination that it was partly full of the moisture from the plant. Again in 1908, in an absolutely gameless and practically waterless country, he observed *Glossina morsitans* feeding on a piece of sugar-cane at a point where the pith was exposed. He attempted to catch this fly, but unfortunately failed to do so. Taute (1912) investigated the point experimentally in the following manner. Two hundred tsetse-flies which had been fed daily on blood for a period of two months were starved for five days, and subsequently were given an opportunity of feeding on small pieces of mango fruit. Taute records that in three cases *Glossina morsitans* buried its proboscis completely in the fruit and remained in this position for several minutes, but definite sucking did not take place. One of the flies was killed and dissected immediately after this operation. Neither in the lumen of the proboscis nor in the oesophagus or remainder of the alimentary canal could the smallest trace of mango juice be recognised.

The work of Carpenter (1912-13), however, affords strong support to the view that *Glossina palpalis* does take up other food than blood. Carpenter examined the intestinal contents of a large number of freshly caught *G. palpalis*, and found in a proportion of them small fragments of tissue of an obviously vegetable origin, e.g., pieces of vegetable parenchyma, starch grains, pieces of alga, and a minute fungus.

Apart from the solitary instance recorded by Maugham, there appears to be no direct evidence that tsetse-fly will imbibe anything other than vertebrate blood, and, moreover, the evidence offered by him is by no means convincing, as he omits to state the manner in which he examined the fly or how he detected that it was partly filled with moisture from the plant. Stuhlmann affirms that *Glossina* lives exclusively on living blood, and that they refuse to take up shed blood, water or syrups; and Degen attempted to feed them on fruit, saccharine fluids, meat, etc., without success. Rodhain, Pons, Vandenbranden and Bequaert (1912) observe that it is generally admitted that tsetses cannot engorge themselves with blood unless they obtain it directly from the capillaries in which the



fluid is maintained under a definite pressure. Leaving pressure out of the question for the moment—Rodhain and his collaborators have since shown that this is not an essential factor—all experimental evidence shows that *Glossina* are unable to imbibe shed blood. In the experiments described below we have demonstrated that they will not absorb certain other fluids offered to them in open vessels.

Fourteen flies which had been starved for 48 hours were placed in each of two cages. In one of these was a petri dish filled with the following solution:—

Sodium chloride...	...	...	0.9 g.
Sugar	...	...	2.0 g.
Neutral red	...	...	0.1 g.
Water	...	...	100 c.c.

and in the other a petri dish containing the following:—

Methylene blue	...	...	0.05 g.
Sodium carbonate	...	...	0.025 g.
Water	...	...	100 c.c.

Small twigs and leaves were floated on the surface of the solutions. The flies were dissected as they died; all those that were still alive at the end of 48 hours were killed and examined. There was no indication that any of the flies had imbibed fluid. These results are conclusive because, as will be seen later, solutions of neutral red and of methylene blue in the concentrations used, if taken up by *Glossina*, stain the tissues densely. Such work as we have done, therefore, confirms the generally accepted view that *Glossina* is unable to imbibe exposed fluids directly.

The work of Rodhain and his collaborators showed, however, that tsetses can suck up citrated blood through a membrane consisting of the freshly removed skin of a mouse. We made use of this observation to prove that not only will tsetses take up citrated blood through such a membrane, but that they will also imbibe various other fluids. The apparatus used by us was a slight modification of that figured and described by Rodhain. It consisted of a short glass cylinder about three-quarters of an inch in diameter, the lower end of which was closed by a cork through which the shorter limb of an U-shaped piece of glass tubing passed; the upper end of the cylinder was covered by the membrane. The pressure of the fluid in the cylinder was indicated by its level in the longer limb of the glass tube.

In our first series of experiments flies were fed on defibrinated goat-blood through a membrane of fresh rat's skin. It was observed that (within the limits of the experiment) the pressure made no difference to the manner in which the flies engorged themselves. They distended themselves without the least difficulty at pressures ranging between + or - 60 mm. of blood. It was further noted that full distension of the flies occurred at least as rapidly and regularly as when the flies were allowed to feed on live rats. On the

TABLE IV.—Giving the results of feeding *G. palpalis* on defibrinated blood and various dilutions of this with normal salt solution, through a membrane consisting of rat skin

No.	Sex	Membrane	Nature of fluid	Result	Remarks
1	♂	Rat skin ...	Undiluted goat blood	Complete distension	Immediately
2	♂	" ...	"	"	"
3	♂	" ...	"	"	"
4	♂	" ...	"	"	"
5	♂	" ...	"	"	"
6	♂	" ...	"	"	"
7	♂	" ...	50 % goat blood ...	"	"
8	♂	" ...	"	"	"
9	♂	" ...	"	"	"
10	♂	" ...	25 % goat blood ...	"	"
11	♂	" ...	"	"	"
12	♂	" ...	"	"	"
13	♂	" ...	10 % goat blood ...	"	"
14	♂	" ...	"	Partial distension ...	After 15 minutes
15	♂	" ...	"	"	"
16	♂	" ...	10 % rat blood ...	Complete distension	Immediately
17	♂	" ...	"	"	"
18	♂	" ...	"	"	"
19	♂	" ...	5 % goat blood ...	No visible distension	After 15 minutes goats' cells seen in anterior gut
20	♀	" ...	"	"	After 15 minutes no goats' cells seen in anterior gut
21	♀	" ...	5 % rat blood ...	"	After 15 minutes rats' cells seen in anterior gut
22	♀	" ...	"	Partial distension ...	After 15 minutes
23	♂	" ...	"	No visible distension	After 15 minutes red cells seen in anterior gut
24	♂	" ...	"	Marked distension ...	After 15 minutes

other hand the flies attacked the membrane with less alacrity than they do the skin of the living animal. Nevertheless, many flies settled on and pierced the membrane at once, and, as a rule, a little patience was sufficient to induce a large proportion of the flies to feed. An experiment was conducted to determine the effect of diluting the defibrinated blood with salt solution in various degrees; the results are given in Table IV. It was found that *Glossina palpalis*

engorged itself with the following dilutions, viz., 50 per cent. blood, 25 per cent. blood, and 10 per cent. blood, but the dilution consisting of 5 per cent. blood and 95 per cent. salt solution was not taken up by the tsetses with the same rapidity and regularity as the other solutions. Of the six flies which were offered the last dilution of blood, only two partially distended themselves; in the other four no distension was observed, but in three of these red cells were seen on dissection.

Physiological salt solution alone was then tried, but although the flies attacked the membrane with the same eagerness as before, no visible distension was noted, except in one instance where the fly appeared to become partially engorged. Tsetses frequently inserted their proboscis and appeared to endeavour to feed; in some instances the membrane was pierced probably at least 100 times with apparently no result. We shall return later to the question whether these flies had actually taken up any salt solution, and simply note here that no distension comparable to that observed when defibrinated blood is offered took place.

We next turned our attention to the question of what element of the blood proves so attractive to the fly that it engorges itself with this fluid to such a marked degree. Fresh defibrinated goat-blood was centrifugalised, and the red cells separated from the plasma; the red cells were then washed free from plasma with normal sodium chloride solution and a 50 per cent. suspension of them made in salt solution—a concentration which corresponds approximately to that of red cells in normal goat-blood. It was found that *Glossina palpalis* engorged itself readily with the red cell suspension. By the addition of normal salt solution, suspensions containing 25 per cent., 10 per cent. and 5 per cent. of red blood cells were made. The results of offering these to the flies are given in Table V. The tsetse engorged themselves completely with all the higher concentrations, but in the case of the 5 per cent. suspension of red blood cells only one of three flies became completely distended, whilst in the other two no distension was observed, although red cells were found in the gut on dissection.

Attempts were made to feed a number of *Glossina palpalis* on fresh defibrinated plasma. The plasma was obtained by shaking up goat's blood in a bottle with beads. After separation of the fibrin

by straining through gauze, the red cells were thrown down by centrifugalisation and the plasma siphoned off. Plasma thus prepared is always of a slightly reddish tint, owing to a certain amount of damage to red cells during the process of defibrination, but the amount of haemoglobin dissolved is so small as to be

TABLE V.—Giving the results of feeding *G. palpalis* on suspensions of red blood cells of various concentration through a membrane of rat skin

No.	Sex	Membrane	Nature of suspension	Result	Remarks
1	♀	Rat skin ...	50 % goat erythrocytes	Complete distension	Immediately
2	♀	" ...	"	"	"
3	♀	" ...	"	"	"
4	♀	" ...	"	"	"
5	♀	" ...	"	"	"
6	♀	" ...	"	"	"
7	♀	" ...	25 % goat erythrocytes	"	"
8	♀	" ...	"	"	"
9	♀	" ...	10 % goat erythrocytes	Partial distension ...	After 15 minutes
10	♀	" ...	"	"	"
11	♀	" ...	"	Complete distension	Immediately
12	♀	" ...	5 % goat erythrocytes	"	After 15 minutes
13	♀	" ...	"	No visible distension	After 15 minutes goats' erythrocytes seen in gut
14	♂	" ...	"	"	"

TABLE VI.—Giving the results of feeding *G. palpalis* on defibrinated plasma through a membrane of rat skin

No.	Sex	Membrane	Nature of fluid	Result	Remarks
1	♂	Rat skin ...	70 % plasma and 30 % normal saline	No visible distension	After 15 minutes
2	♀	" ...	"	Slight distension ...	"
3	♀	" ...	"	No visible distension	"
4	♀	" ...	"	"	"
5	♀	" ...	"	"	"
6	♀	" ...	"	Almost complete distension	"
7	♂	" ...	"	No visible distension	"
8	♀	" ...	"	Complete distension	"

practically negligible. Two of eight flies, all of which had made repeated efforts to feed, succeeded in completely engorging themselves. In only one of the remaining six was slight distension noticeable. The results are given in Table VI.

From these experiments it is apparent that the most attractive element in the blood is the red corpuscle. In order to carry the matter further, the washed red cells of the goat were laked by the addition of three parts of distilled water to two parts of red cells. This solution was offered to three *Glossina palpalis*, all of which quickly and completely engorged themselves, thus proving that the integrity of the red corpuscles is not an essential factor. An attempt was then made to separate the red cell stromata from those constituents of the corpuscle which are soluble in water. For this purpose sufficient sodium chloride was added to the laked red cell solution to render it isotonic, and the stromata were subsequently precipitated by prolonged centrifugation. Unfortunately, we did not succeed in obtaining complete separation of the stromata, as we had no high power centrifuge at our disposal, and owing to the minute size of the goat's erythrocytes precipitation of the stromata is a matter of no small difficulty. Nevertheless, although complete separation was not obtained, a considerable fraction of the total amount of stromata was precipitated and a solution of goat's haemoglobin, fairly free from solid matter, was obtained. The resulting solution contained approximately as much haemoglobin as that found in a 40 per cent. suspension of goat's red cells.

Four flies to which this solution was offered rapidly and completely distended themselves. The solution was then diluted with an equal volume of physiological saline. It was found that the six flies to which this solution was offered did not feed with the same avidity as did those which were offered the more concentrated solution; one became completely distended, three others partially, whilst in two no distension occurred.

Having thus determined that *Glossina palpalis* feeds readily on fresh haemoglobin solution, further experiments were performed with solutions made from the crystallised haemoglobin of commerce (Grübler's dried haemoglobin). Solutions made from this preparation are of a brownish-red colour, and give rather indistinct absorption bands of oxyhaemoglobin. The strength of the solution used was between 1 and 2 per cent. Four of the five flies to which this solution was offered became partly distended, whilst in the case of the other, although no distension was visible, the solution was seen in the gut on dissection. None were found to engorge

themselves completely in a manner comparable to that seen with haemoglobin solution made from fresh blood. Details are given in Table VII.

Owing to the lack of suitable apparatus, we were unable to determine the result of offering to *Glossina palpalis* the washed stromata derived from red blood cells.

TABLE VII.—Giving the results of feeding *G. palpalis* on haemoglobin solutions of various concentration through a rat skin membrane

No.	Sex	Membrane	Nature of fluid	Result	Remarks
1	♀	Rat skin ...	40%* solution of goat haemoglobin in 0.9% NaCl	Complete distension	Immediately
2	♀	" ...	"	"	After 5 minutes
3	♀	" ...	"	"	Immediately
4	♀	" ...	"	"	"
5	♀	" ...	20%* solution of goat haemoglobin in 0.9% NaCl	Partial distension ...	After 15 minutes
6	♀	" ...	"	Slight distension ...	"
7	♀	" ...	"	Almost complete distension	"
8	♀	" ...	"	No visible distension	"
9	♀	" ...	"	Slight distension ...	"
10	♀	" ...	"	No visible distension	"
11	♀	" ...	1—2% solution of dry crystalline haemoglobin	Partial distension ...	"
12	♀	" ...	"	"	"
13	♀	" ...	"	"	"
14	♀	" ...	"	No visible distension	After 15 minutes Hb. solution seen in anterior gut
15	♂	" ...	"	Slight distension ...	After 15 minutes

\* By this is meant that the solutions contained respectively as much haemoglobin as do 40 and 20 per cent. suspensions of goat red blood cells.

Summarising the results of these experiments, we find that *Glossina palpalis* feeds with avidity, through rat's skin, on fresh defibrinated blood and also on suspensions (50 to 5 per cent.) of washed red blood cells in normal saline, and on solutions containing as much dissolved haemoglobin as is present in 40 and 20 per cent. suspensions of red blood cells. Defibrinated plasma does not appear to have the same attraction for them, nor does sodium chloride solution alone, but the latter, containing a small proportion of the dried haemoglobin of commerce in solution is to a certain extent

taken up. Although these experiments are not quite so conclusive—especially as regards separation of the constituents of the red blood cells which are soluble and insoluble in water—as could be desired, nevertheless, we consider they suggest strongly that the element in the blood which is attractive to *Glossina palpalis* is that fraction of the erythrocyte which is soluble in water, most probably haemoglobin.

Having completed these observations on blood, we decided to make use of the same technique with a view to determining whether *Glossina palpalis* will take up other solutions through fresh rat-skin. Reference has already been made to the fact that when sodium chloride solution was offered, although the flies repeatedly pierced the membrane, they did not appear to take up any of the fluid. At all events, no distension was noticed as a rule, although in one instance partial engorgement did apparently occur; it was by no means easy to state definitely whether or not any of the solution had been imbibed, as small quantities of a clear fluid cannot be recognised in the gut of the fly. In order to determine the point, the solution must be coloured with some dye that can be readily recognised. For this purpose methylene blue, neutral red and fuchsin were used. It was found that when physiological saline, containing these dyes in solution, was offered to *Glossina palpalis* small quantities were taken up by the flies in a proportion of the instances. Dissection showed that the intestinal tract was deeply stained, red or blue according to the dye used. In some cases the stain had spread to the salivary glands and to the fat bodies. In fact, so deeply were certain flies coloured that even before dissection the dye could be recognised through the integument of the abdomen. After obtaining this information various other solutions were offered to *Glossina palpalis*, details of which are given in Table VIII. It is interesting to note that three flies became completely engorged with 0.9 per cent. sodium chloride solution to which had been added about 5 per cent. of cane-sugar and a little neutral red. Another fly became partially distended on a 25 per cent. solution of glycerine in water coloured with methylene blue.

These observations demonstrate that under certain conditions *Glossina palpalis* will imbibe and even completely distend itself with fluids other than blood. Although in all the experiments just described the membrane used was the freshly removed skin of a rat

or rabbit, nevertheless we found that this tsetse will feed through other membranes, e.g., sheep's bladder, peritoneal tissue and membrane composed of a thin sheet of rubber. As regards the last, one fly out of five completely distended itself with defibrinated blood through this membrane. Although the flies had not the slightest

TABLE VIII.—Giving the result of feeding *G. palpalis* on various fluids through a membrane of skin

No.	Sex	Membrane	Nature of fluid	Result	Remarks
1	♀	Fresh rabbit skin ...	Methylene blue solution*	No visible distension, but abdomen of bluish tinge	Anterior and mid-gut blue. Posterior gut green. Salivary glands, malpighian tubules and fat bodies blue
2	♂	Same rabbit skin 24 hours old	"	No visible distension	"
3	♂		"	"	No staining seen on dissection
4	♀		"	"	"
5	♀		"	"	Proventriculus and anterior gut blue
6	♂	"	"	No visible distension, but abdomen of bluish tinge	Whole intestine (except rectum), salivary glands, malpighian tubules and fat bodies deeply stained
7	♀	"	Neutral red .1% solution in water	No visible distension	Anterior gut red
8	♂	Fresh rat skin ...	Fuchsin .1% in .9% NaCl solution	Slight distension	Proventriculus and anterior gut pink
9	♀				
10	♀				
11	♂				
12	♀	"	Neutral red .1% solution in water + sugar	Considerable distension	Whole gut (except rectum), salivary glands, malpighian tubules and fat bodies deeply stained
13	♀				
14	♀				
15	♀				
16	♂	"	"	Complete distension	"
17	♂	"	Methylene blue solution + sugar	No distension ...	Anterior and mid-gut deep blue
18	♂	"	"	"	"
19	♂	"	"	"	Slight staining of anterior gut
20	♂	"	"	"	No staining
21	♂	"	75% methylene blue solution + 25% glycerine	"	"
22	♂	"	"	Partial distension ...	Anterior and mid-gut and also salivary glands and fat bodies stained
23	♂	"	"	"	Anterior gut blue

\* The methylene blue solution used was the weak solution employed in Romanowsky's stain; its formula is given on page 369.

difficulty in piercing the rubber, they did not seem able in the majority of cases to imbibe blood through it; possibly owing to the extremely elastic nature of the rubber the lumen of the proboscis was occluded, thus preventing the passage of fluid through it.



The practical point that we have to decide is, does *Glossina* in nature obtain food other than blood? Although this question is not answered by the experiments described above, yet the results obtained are very suggestive. They show that under favourable conditions *Glossina* will take up solutions of vegetable origin, such as sugar and water. Apparently one essential is that the fluid should be presented enclosed by a membrane. The reason for this

TABLE IX.—Giving the results of feeding *G. palpalis* on blood through membranes other than freshly-removed skin

No.	Sex	Membrane	Nature of fluid	Result	Remarks
1	♂	Rabbit skin removed 24 hours previously and partially decomposed	Defibrinated goat blood	Complete distension	Immediately
2	♀	"	"	"	"
3	♀	"	"	Partial distension ...	After 15 minutes
4	♀	Ox bladder ...	"	Complete distension	Immediately
5	♀	"	"	"	"
6	♀	"	"	Partial distension ...	After 15 minutes
7	♀	"	"	Slight distension ...	"
8	♀	Peritoneal membrane, ox	"	Partial distension ...	"
9	♀	"	"	Slight distension ...	"
10	♀	"	"	"	"
11	♀	Thin rubber sheeting	"	"	"
12	♀	"	"	Complete distension	"
13	♀	"	"	No visible distension	"
14	♀	"	"	"	"
15	♀	"	"	"	"
16	♀	"	"	"	"

is not that the flies require the fluid to be at a positive pressure before they can imbibe; it is possibly a mechanical difficulty, the fly being unable to take up fluid unless its proboscis is buried in the membrane or tissue. The experiments show, moreover, that even when the proboscis is inserted into a membrane, it does not necessarily follow that the fluid enclosed will be imbibed to any considerable extent. The results obtained prove most definitely that *Glossina palpalis* exhibits a preference for certain fluids, especially blood, red cells and haemoglobin. The physical character of the fluid does not, within limits, appear to matter. Defibrinated blood, a 50 per cent. suspension of red cells in saline, and a solution obtained by laking two parts of washed red cells with three of distilled water, are taken

up with equal readiness, whilst plasma and normal salt solution are only imbibed in small quantities as a rule.

Turning to the question whether *Glossina* takes up vegetable juices in nature, we may state at once that we have not had the opportunity of deciding the point absolutely. *Glossina* of both sexes were frequently seen to plunge their proboscis into bananas, oranges and mangoes which had had their skin removed, and also into the skin itself. The insertion of the proboscis was repeated again and again, and the organ often remained buried in the fruit for a minute or more; the whole procedure certainly gave one the impression that the flies were endeavouring to obtain food from the fruit. Furthermore, we noticed that *Glossina palpalis* will pierce leaves placed on the surface of a fluid with the object apparently of imbibing the fluid below.

Unfortunately, we had not time to extend our observations on this subject, but in view of the results already detailed, and of the observations of Carpenter who found vegetable tissue in a proportion of wild *G. palpalis* dissected by him, we consider that it is highly probable that tsetse do take up food of vegetable nature. The frequency with which a hungry fly will insert its proboscis, or at least attempt to do so, into practically any object presented—they can even be seen trying to pierce the glass vessel in which they are kept when no skin is in immediate contact with the glass—suggests that, in the absence of blood, they are on the look out for other food, for there is every reason to believe that *Glossina* recognises when it is in the presence of the skin of a living animal.

Assuming *Glossina palpalis* cannot reproduce itself or live for any long period in the absence of blood, it is possible that food of a vegetable nature may suffice to enable it to exist in a locality which for a certain period in the year is denuded of vertebrates.

### CONCLUSIONS

1. About eight per cent. of the wild *G. palpalis* in this district contain recognisable red blood cells—seven per cent. of mammalian origin and one per cent. nucleated red cells of unknown origin.
2. Seventy-two hours after *G. palpalis* had completely distended itself on rat's blood recognisable red cells could no longer be found

in its intestine; after being fed on a fowl nucleated red blood cells could be recognised in 40 per cent. of cases at the end of a similar period. The flies were kept at a temperature of 80°-86° F.

3. Neither shed blood nor other fluid which is exposed (not covered by a membrane) can be imbibed by *G. palpalis*.

4. *G. palpalis* can take up through a membrane of fresh skin not only blood and various dilutions of it with normal saline, but also suspensions of red blood cells in normal saline, and solutions of haemoglobin (both freshly made from red blood cells, and the dried crystalline preparation of commerce) in distilled water.

5. Fluids other than blood such as solutions of sugar, sodium chloride, and glycerine, in water containing a small quantity of a dye (methylene blue, neutral red or fuchsin) are also taken up through a membrane of fresh skin by *G. palpalis*, but not so quickly or so readily as is blood.

6. *G. palpalis* exhibits a definite selective taste for the various fluids presented to it under the membrane; blood, red cells, and haemoglobin solution being much preferred. The attractive element in the blood is the fraction of the red cells soluble in water, probably haemoglobin.

7. *G. palpalis* which had been starved for a day or two can often be seen to insert the proboscis repeatedly into oranges, bananas or other fruits which may be offered them.

8. We are of opinion that *G. palpalis* in nature may under certain conditions take up fluid other than blood.

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## THE CULTIVATION OF THE LEPROSY BACILLUS

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In an article entitled 'Leprosy: a perspective of the results of experimental study of the disease,' by Bayon, and published in the *Annals of Tropical Medicine*, Vol. IX, the statement is made on page 29 that 'Fraser and Fletcher discarded all diphtheroids because of their ubiquity.' It is unfortunate that he has quoted our work incorrectly. In the *Lancet*, Vol II, 1913, we published an article entitled 'The *Bacillus leprae*: has it been cultivated?' and on page 920, under the head of contaminating micro-organisms, we state that 'In common with other workers we have isolated diphtheroid organisms, but these are ubiquitous and demand no special consideration.' At the time that article was written, our investigations had led us to form the opinion that the diphtheroids were contaminators, and had no genetic relationship with the leprosy bacillus; further investigations carried out during the past two years have confirmed that opinion.

If we had confined our observations to the results of one or two experiments, as has been done by so many workers, we might, perhaps, like Bayon, have formed the opinion that the diphtheroids were related to the leprosy bacillus, but to do so we should have had to reconcile or, since that was impossible, to ignore some remarkable discrepancies.

It seems a comparatively simple operation to reflect the skin from a leprous nodule and to excise a portion of the subcutaneous tissue free from contamination, but only those who have performed the operation a sufficient number of times can be aware of the pitfalls.

Cultures of diphtheroids were obtained only in our early experiments; in these a nodule of tissue rich in leprosy bacilli was excised, and from twenty to thirty tubes of culture media were inoculated

with portions of it. On one tube, perhaps two tubes, a culture of a diphtheroid would be obtained. As each tube was inoculated with an enormous number of leprosy bacilli from the same nodule, it is impossible to believe that only in one or two parts of that nodule was there an acid-fast bacillus capable of proliferation under saprophytic conditions into a diphtheroid. If every tube inoculated with portions of the nodule had developed a culture of a diphtheroid the opinion formed might have been quite different, but the sporadic occurrence of a diphtheroid is quite in accordance with our results obtained in the isolation of diphtheroids from other parts of the body, and we believed the correct interpretation of these experiments to be that the nodule was contaminated, or had become contaminated from the skin, with one or two diphtheroid bacilli which proliferated on the culture media.

In that belief we extended our investigations and improved our technique, avoiding the use of all disinfectants for the skin. We were then able to excise leprous nodules free from contamination, and from which a culture of no organism could be obtained on any medium.

We have now excised material from fifty-two non-ulcerating nodular cases of leprosy, a number far in excess of that recorded by any other worker, and have employed the media of every claimant to success, but have not substantiated the claims of any of them.

Our work has been carried out over a period of three and a half years. The only conclusions, which that work permits, are that the leprosy bacillus has not been cultivated and that the diphtheroids and other organisms are merely contaminators.

Elsewhere we (1914) have dealt with Kedrowsky's culture of an acid-fast bacillus which Bayon claims to be the leprosy bacillus. Our experiments have led us to conclude that there is no evidence that the acid-fast bacillus of Kedrowsky is the leprosy bacillus.

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## THE RESERVOIR OF THE HUMAN TRYPANOSOME IN SIERRA LEONE

[*Being the Third Report of the Thirty-second Expedition of the  
Liverpool School of Tropical Medicine, 1914-1915.*]

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No time was spent in examining the native population for the incidence of trypanosomiasis, but two cases of sleeping sickness—a woman and her child—were brought to our notice by Drs. Young and Butler of the West African Medical Service. These cases which were extremely chronic were discovered accidentally, one or two trypanosomes being seen during the examination of the blood for malarial parasites. The patients lived in Lumley, a native village situated at the junction of the Cape Lighthouse Peninsula with the mainland, about a mile and a half from Hill Station and four miles from Freetown. Trypanosomes were first seen in the mother (Catherine Macauley) about the beginning of 1914. She received a single treatment with salvarsan, and subsequently returned to Lumley. When seen by us on December 7th, 1914, she was in good condition. According to Dr. Young, no definite change had occurred during the year. The only symptoms were headache and a feeling of weakness. She had one or two slightly enlarged glands in the posterior triangles. Several careful examinations of the peripheral blood and gland juice failed to reveal trypanosomes. On the day she was first seen by us (December 7th) two rats were inoculated with blood and one with gland juice. After 28 days one of the two rats inoculated with the blood was found to be infected with trypanosomes. The other two rats were kept under observation until they died, on the 59th and 67th days respectively; trypanosomes were never found in them. There was no change in

the condition of the patient when she was last seen by us on February 24th, 1915. On December 16th, 1914, she brought her son, aged 8, to the hospital suffering with fever. On examination of a stained preparation of his blood by Dr. Butler, malarial parasites were found and also a single trypanosome. On December 19th the boy was seen by us. He complained of pain over the spleen, which was enlarged, reaching to within one inch of the umbilicus. There were a few shotty glands in the neck, axillae and groins. Malaria parasites were still present in the blood, but no trypanosomes were seen. Two rats were inoculated with 1 c.c. of the peripheral blood. Neither of these animals became infected; one died on the 52nd day afterwards, and the other was still alive on the 90th day when we left the Colony. The blood of this boy was examined by us on several other occasions, but trypanosomes were not seen.

The history of these two cases shows that sleeping sickness in Sierra Leone is exceedingly chronic, and very difficult to recognise. Trypanosomes can be found only occasionally in the peripheral blood; gland puncture of the mother was negative, whilst the boy had no glands which were puncturable.

The question whether there is any reservoir of the human trypanosome in Sierra Leone other than man is of importance, and is one which we decided to investigate. By the term *Reservoir* we mean the source from which *Glossina* derives its infection. Kinghorn and Yorke (1912) have shown that the chief reservoir of the human trypanosome of South Central Africa (*T. rhodesiense*) is the antelope and not man. The antelope differs from man in being tolerant of the infection; unlike man, in whom the disease runs an acute course, it is able to harbour *T. rhodesiense* in its blood for long periods without exhibiting signs of the disease. Both for this reason and because the antelope exists in enormous numbers in many parts of South Central Africa, coinciding in its distribution with the ubiquitous *G. morsitans*—the fly responsible for the transmission of sleeping sickness in this region—it constitutes a much greater and more reliable reservoir of the human trypanosome than does man. In Sierra Leone, however, we have to deal with quite a different state of affairs; sleeping sickness in this part of Africa is an extremely chronic infection. In striking contrast to



cases infected with *T. rhodesiense*, those infected with the human trypanosome of the West Coast may live for many months, or even years, without exhibiting any serious indication of the disease. For this reason, therefore, man in Sierra Leone is a more constant and dependable reservoir of the virus of sleeping sickness than he is in Rhodesia or Nyasaland. Moreover, in the Colony of Sierra Leone, and in most portions of the Protectorate, large game is comparatively rare. Again, the most prevalent tsetse-fly in Sierra Leone is *G. palpalis*; owing to its predilection for water-courses its distribution is limited much more closely to the haunts of man than is that of the ubiquitous *G. morsitans*. In Sierra Leone, therefore, we must conclude that of large game and man, the latter constitutes by far the more important reservoir of the human trypanosome.

There is, however, another possible reservoir of the human trypanosome which must not escape attention, that is domestic stock. The Sleeping Sickness Commission of the Royal Society (1910) examined seventeen cattle in Uganda, and found one cow infected with *T. gambiense*. This element does not exist in Rhodesia or Nyasaland, as quite apart from *T. rhodesiense* cattle are unable to thrive to any extent in the presence of large numbers of *G. morsitans*. Although cattle are not bred in most portions of Sierra Leone, yet they are imported from French Senegal in large numbers, and gradually find their way down to Freetown for slaughter.

Both in the Protectorate and in the Colony the blood of a number of domestic animals—143 animals, 7 goats, 7 sheep and 10 dogs—was examined. As will be seen in a subsequent paper, the trypanosomes most commonly found were *T. vivax* and *T. congolense*. One ox, however, was found to be infected with a trypanosome which we are unable to distinguish from that infecting man. The ox in question was one of a herd of 90 Government cattle in a *Warri* at Batkanu. All the animals in this herd had originally come from French territory, but they had been in the Protectorate for various periods before being bought by the Government. The animal in which this trypanosome was found appeared to be in perfect health; direct examination of its peripheral blood was negative. The trypanosome was discovered fortuitously owing to the fact that this ox happened to be one of nine chosen at random

from the herd for the purpose of having their blood inoculated into rats. Trypanosomes were seen in the peripheral blood of the rat fourteen days after inoculation. The parasite was a polymorphic trypanosome, it did not exhibit posterior nuclear forms, and was indistinguishable morphologically from the trypanosome isolated from the human case of trypanosomiasis.

During the last six months the strain has been maintained by passage through rats. The course of the infection in these animals is exceedingly chronic, and certain rats failed to become infected. For purpose of comparison, the result of inoculation of rats and guinea-pigs with this strain and with that obtained from man are given in tabular form.

There is a striking similarity in the pathogenicity of the two strains in rats. The virulence of both is but slight, and is in marked contrast to that of the other common polymorphic trypanosomes—*T. pecaudi* or *T. rhodesiense* vel *ugandae* (*T. brucei*, Uganda). On consulting the results of inoculation of rats with *T. gambiense* direct from man (Yorke, 1910) one cannot fail to observe their striking similarity to those set forth in the above table. Macfie (1914) records that he failed to infect rats with the Nigerian strain of the human trypanosome (*T. nigeriense*). A guinea-pig infected with *T. nigeriense* was, however, sent by Macfie to us at Runcorn, and from it we succeeded without much difficulty in infecting a number of rats. The course of the infection in these animals was exceedingly chronic often ending in recovery; some failed to become infected.

We are of opinion, therefore, that this polymorphic trypanosome from the ox is identical with that infecting man in West Africa, in other words, that it is *T. gambiense*.

The discovery of the human trypanosome in the ox is important. It shows that domestic stock may serve as a reservoir of *T. gambiense*. Before we can form any opinion as to the relative importance of this reservoir we must have information on the following two points:—

- (1) Do the animals harbour the parasite in their blood for long periods without exhibiting signs of disease, or does the infection run an acute course?
- (2) What percentage of the animals is infected?

TABLE I.—Giving results of inoculation of rats with the human strain of *T. gambiense*

No. of Animal	Animal from which inoculated	Incubation in days	Length of life in days	Remarks
Rat 1a ... ..	Patient's blood ...	28	156	
„ 1b ... ..	„ „ ...	—	—	Did not become infected
„ 10a ... ..	Rat 1a ... ..	8	88	
„ 10b ... ..	„ 1a ... ..	13	—	Alive on 171st day. Trypanosomes last seen on 50th day
„ 37a ... ..	„ 10a ... ..	4	29	
„ 37b ... ..	„ 10a ... ..	—	—	Did not become infected
„ 51a ... ..	„ 10a ... ..	—	—	„ „
„ 51b ... ..	„ 10a ... ..	—	—	„ „

TABLE II.—Giving results of inoculation of rats and guinea-pigs with the ox strain of *T. gambiense*

No. of Animal	Animal from which inoculated	Incubation in days	Length of life in days	Remarks
Rat 24 ... ..	Ox VIII ... ..	14	40	
„ 31 ... ..	Rat 24 ... ..	7	—	Alive on 160th day. Trypanosomes last seen on 24th day
„ 47a ... ..	„ 24 ... ..	10	39	
„ 47b ... ..	„ 24 ... ..	10	—	Alive on 139th day. Trypanosomes last seen on 67th day
„ 49a ... ..	„ 31 ... ..	—	—	Did not become infected
„ 49b ... ..	„ 31 ... ..	—	—	„ „
„ 50 ... ..	„ 47b ... ..	10	63	
Guinea-pig 52 ...	„ 50 ... ..	—	—	Did not become infected
Rat 53a ... ..	„ 50 ... ..	18	—	Alive on 46th day
„ 53b ... ..	„ 50 ... ..	18	—	„ „
Guinea-pig 54 ...	„ 53a ... ..	—	—	Did not become infected
Rat 55 ... ..	„ 53a ... ..	13	—	Alive on 31st day

As regards the first point, the ox from which the trypanosome was obtained appeared in perfect health, and a report received four months later states that there is still no indication of disease. Regarding the second point, we have as yet no evidence to show what proportion of the cattle in Sierra Leone are infected with *T. gambiense*. If, as appears probable, cattle prove to be tolerant of this parasite and can harbour it in their blood for long periods without detriment to health, it will be no easy matter to determine how many animals are infected. As already mentioned, the parasite was discovered largely by chance; although a direct examination of the peripheral blood of each of the 90 animals in the Warri was made, subinoculation into rats was done in nine instances only. Furthermore, it must be borne in mind that rats not infrequently fail to become infected after inoculation of blood containing *T. gambiense*. It will, therefore, probably be at least as difficult to determine the percentage of cattle infected as it is to ascertain the number of infected human beings.

Before leaving the subject, it is interesting to refer to the observations recorded by Macfie. This investigator holds that in the Eket district man does not constitute a reservoir of sleeping sickness, and that the reservoir is still unknown. The evidence on which he bases this assumption is, in our opinion, unconvincing. He writes 'The extreme rarity or complete absence of trypanosomes from the peripheral blood of all the human cases examined, the rarity of the parasites even in the gland juice, and the difficulty experienced in infecting animals by inoculation, suggest that it must be a very exceptional occurrence for a tsetse-fly to become infected by feeding on these cases. . . . It seems, therefore, that the human infections must be dependent on some other cycle of development, including an insect and some so far unidentified animal host, the reservoir of the disease. The ordinary development of the trypanosome may take place in these two hosts. The insect, infected from the animal host may, however, be capable of infecting human beings; but the disease may be so modified in them that they are incapable of handing on the infection any further.' This argument appears fallacious. In view of the fact that trypanosomes were not found in the peripheral blood they were doubtless scanty, but it does not by any means follow that they were absent. Nor

does the failure to infect animals by inoculation of blood materially assist the argument, since Macfie himself found what has frequently been pointed out by previous workers that rats and guinea-pigs often fail to become infected after the injection of blood known to contain *T. gambiense*. Macfie records that all his attempts to infect rats with blood containing the trypanosomes were unsuccessful; further, of seven guinea-pigs inoculated with gland juice actually containing trypanosomes only one became infected.

This comparative insusceptibility of rats and guinea-pigs to human trypanosomes of the West Coast shows that we cannot safely regard a negative result obtained by inoculation as evidence that trypanosomes were not present in the blood inoculated. It is, therefore, inadmissible to argue, as does Macfie, from the negative results of microscopic examination of the blood and of inoculation of guinea-pigs and rats that *G. palpalis* cannot become infected from human beings. In 5 c.c. of the blood of one of these patients there may be sufficient trypanosomes to infect many flies, and yet insufficient to infect a particular guinea-pig or rat.

Briefly, Macfie's view appears to be this. Man is not a reservoir of the virus of sleeping sickness because *Glossina* cannot become infected from him. He postulates an unknown vertebrate reservoir of the virus and an insect host, in which the ordinary development of trypanosomes takes place. The insect may infect man but cannot become infected from him, but only from the hypothetical vertebrate reservoir.

For the reasons given above, we consider that Macfie has failed to show that *Glossina* cannot become infected from man. In our opinion, there is no evidence which would lead us to believe that man does not constitute a most important reservoir of the human trypanosome of West Africa. Nevertheless, the fact that the human trypanosome has been discovered in an ox indicates that domestic stock also forms a reservoir, the extent of which we are at present unable to estimate, but the existence of which must be recognised when prophylactic measures are contemplated.

### CONCLUSIONS

1. The human trypanosome, *T. gambiense*, has been found in an ox in Sierra Leone.

2. It is impossible at present to form any conclusion of the extent to which domestic stock may harbour this parasite. In all probability, it will prove to be at least as difficult to recognise the infection in cattle as it is in man.

3. The existence in domestic stock of a potential reservoir of *T. gambiense* will have to be taken into account when prophylactic measures are contemplated.

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# SPIROCHAETA BRONCHIALIS, CASTELLANI, 1907, TOGETHER WITH REMARKS ON THE SPIROCHAETES OF THE HUMAN MOUTH

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Liverpool School of Tropical Medicine, to Khartoum, 1913]*

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## PLATE XXXIV

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### I. INTRODUCTION

During the summer of 1913, I had the good fortune to work in the Wellcome Tropical Research Laboratories, Khartoum, having been sent on expedition by the Liverpool School of Tropical Medicine. While there, thanks to the kindness of the Government of the Anglo-Egyptian Sudan and of the Director, Dr. A. J. Chalmers, and Staff of the Wellcome Laboratories, I was able to

undertake researches on human and avian spirochaetes, directing especial attention to bronchial spirochaetosis in man. In this memoir an account is given of some of my researches on the causal agent of the latter disease.

## II. MATERIAL AND METHODS

The material was chiefly obtained from cases of human bronchial spirochaetosis occurring in Khartoum, Omdurman and Kodok. Many of the cases were chronic. They included officers' servants, warders, native policemen, soldiers and household servants. A few cases among Europeans suffering from bronchitic symptoms were studied, and some of them were found to be fresh cases of undoubted spirochaetosis. Among the latter were two cases of almost experimental infection. The total number of cases studied was twenty.

I was fortunate in being able to obtain material from nearly all the cases reported by Drs. Chalmers and O'Farrell (1913). I was also able to examine preparations sent by Dr. J. A. Taylor from Entebbe, Uganda, to whom my best thanks are due. In fact, Dr. Taylor sent me his first preparations for my examination and opinion at the end of May, 1913, just before I left England for the Sudan. *Spirochaeta bronchialis* was also studied in monkeys experimentally infected in Khartoum.

It is with much pleasure that I acknowledge the kindness of Dr. Chalmers and Captain O'Farrell, R.A.M.C.; Major Forrest, R.A.M.C.; Major Carroll, R.A.M.C.; Dr. Christopherson; Dr. Atkey; Dr. Crispin; Captain Stirling, R.A.M.C., and Captain Buist, R.A.M.C., in providing me with material for research. I am especially indebted to the gentlemen first-named for much help while in the Sudan.

The subject is one of great difficulty, and because of this difficulty and the pressure of other work the publication of this paper has been delayed. Also, even now, it is not so fully illustrated as I should have wished. Many more drawings (totalling over 500) have been prepared, but owing to the war these cannot be reproduced without further delay. Hence a selection of what appear to be types of the spirochaetes only are shown on the accompanying plate (XXXIV), the reproduction of which by half-tone process has left something to be desired.



Also, owing to the lack of precise knowledge of spirochaetes found in the human mouth and respiratory passages, attention has been paid to the comparative morphology of these organisms. I have compared the spirochaetes of the mouth found in natives of the Sudan with those found in similar situations in people living in England, especially in cases of pyorrhea alveolaris and of 'gum-boils,' obtained in Liverpool and in Cambridge.

In the Sudan the procedure followed for obtaining material was briefly as follows:—The teeth were examined for caries, as were also the buccal, tonsillary and pharyngeal secretions, with a view to finding spirochaetes. The patient washed his mouth with clean water, and samples of spirochaetes in mouth sputum might also be thus obtained. The throat was then gargled, and usually samples of throat sputum were examined. After this careful washing of the mouth and gargling of the throat, expectoration from the deeper bronchial regions was collected in a sterile petri-dish, and in this *Spirochaeta bronchialis* occurred. The sputum thus collected was examined by dark-ground illumination, and films were made with the aid of a sterile platinum loop for immediate fixation and staining.

Various methods of fixing and staining were tried. Osmic vapour followed by absolute alcohol, Schaudinn's fluid (sublimite-alcohol-acetic) and Bouin's fluid (picro-formol-alcohol-acetic) were used for fixing wet preparations; Bouin's fluid was found most useful. The solutions of Giemsa and Leishman, carbol thionin and gentian violet, were tried, as well as various haematoxylin, such as those of Delafield, Ehrlich and Böhmer. In the hot dry atmosphere of Khartoum, freshly prepared Böhmer's haematoxylin was found to be most useful, and less complicated than iron-haematoxylin, though this was also used. Staining overnight with dilute solutions was found to be more effective in the case of this fragile spirochaete than quick staining with stronger solutions. Unfortunately, gentian violet, so useful for spirochaetes in general, was found in Khartoum to fade quickly, but was very useful when studying mouth spirochaetes in England. Naturally, in the fixation of a spirally wound, flexible organism, some flattening and straightening of the coils is inevitable, the fixed forms thus being less graceful structures than the living ones.

### III. MOVEMENTS

In fresh sputum *S. bronchialis* moves very quickly. Its movement, like that of other spirochaetes, is difficult to analyse. The organism moves forward while turning on its long axis. However, as I have previously described (1907, 1908), such movements may be divided into two components, namely, an undulatory flexion of the body mainly for progression, and a corkscrew or helicoid movement of the body as a whole, due to the winding of the membrane or crista. This latter organella is very narrow and thin, and is a lateral outgrowth of the periplast. It is only seen with difficulty in *S. bronchialis*, and not in some specimens, as it is sometimes so contracted as to appear to be absent. It does not markedly undulate, and should not be called an undulating membrane in spirochaetes, as I pointed out in 1907-8, for it is not directly comparable with the undulating membrane of a trypanosome. It can be seen in some cases in life by the use of the paraboloid condenser. In stained specimens it sometimes appears as a curved line lying along the body when the organism has been fixed during rapid movement (Pl. XXXIV, figs. 2, 22), or as a slightly wavy lateral outgrowth when the organism was killed while moving slowly, the membrane being somewhat relaxed (figs. 10, 16, 36).

The undulatory flexion is responsible for the rippling motion of spirochaetes and the waves seen passing down the body of these organisms.

Slowly moving spirochaetes have few undulations of relatively large amplitudes along their bodies, while quickly moving forms have more numerous small undulations (figs. 19, 23, 28), characters which I also described in 1907-8. Hence the number of coils or waves in a spirochaete is more an index of its rate of motion—as well as of its thickness, which is also a factor—than one of differentiation between various forms. Thus, thin forms are more easily thrown into waves than thick forms, and so the number of waves in a spirochaete cannot be used as a mark of species differentiation as has been attempted by some workers in the past. In spirochaetes exhibiting polymorphism, thin forms represent young individuals, while thick ones are adult forms, as was indicated by me in 1909.

Jerking movements may occur, but not frequently. Spirochaetes are isopolar, and can easily and very quickly return on their own path. Other remarkable appearances are produced by rapid move-

ments in a relatively limited area. Thus, the spirochaete may curve each of its ends inwards in a watchspring-like manner and after a time straighten itself, and then re-form a watchspring coil at either end in the opposite direction. Waves continue to pass along the organism during the movements of the body as a whole. At other times, though more rarely, the organisms intercoil their ends and seem almost to tie themselves in knots, only to extend a few seconds later and swim away in a different direction. The subject of the movements of spirochaetes was fully discussed by me in June and August, 1907, and January, 1908, when dealing with *S. balbianii* and *S. anodontae*, and the same remarks apply to *S. bronchialis* and the spirochaetes of the mouth. There was little evidence of division seen in life.

#### IV. GENERAL MORPHOLOGY

The morphology of spirochaetes is a subject of great difficulty and of no little controversy. In this paper I shall, without prejudice, adopt the generic name *Spirochaeta*, as I cannot at present accept the unconfirmed researches of Zuelzer (1911) on that debatable organism known as *S. plicatilis*. As a result of Zuelzer's researches some workers have proposed to create a number of genera such as *Cristispira* (for the forms found in molluscs), *Spiroschaudinnia* or *Borrelia* (for blood-inhabiting forms), *Treponema*, and the like, but without any clearly and generally accepted generic differences. The divergent descriptions of *S. plicatilis* given by different workers may be clearly seen and contrasted from the account given in Bosanquet's book on Spirochaetes. The so-called axial fibre of Zuelzer is acknowledged to be homologous with the membrane or crista of molluscan spirochaetes. Subsequent researches may lead to a solution of these difficulties.

*Spirochaeta bronchialis* is a delicate organism which frequently stains with difficulty. It exhibits morphological variation (figs. 1-39), due to growth and division. There are long forms (figs. 37-39) and short forms (figs. 1-6); also thick ones (figs. 7, 38) and thin ones (figs. 28, 29, 33), and those of intermediate length (figs. 7-33) and breadth (figs. 25, 34, 35). No detailed account of the morphology of the parasite has yet been published.

The cytoplasm of *S. bronchialis* is almost homogeneous and

shows no vacuoles. In spite of the minuteness and fragility of the organism, chromatin bars can be seen at intervals along the parasite in some of the specimens (figs. 14, 24, 29, 36-39). In stained preparations the observance of these minute bands or rodlets is sometimes aided by the use of stereoscopic eyepieces. When examined fresh these chromatin granules appear as refractile spots, as viewed under dark-ground illumination using a paraboloid condenser. On the other hand, many spirochaetes only show the chromatin dots or granules with difficulty at certain stages of their life-cycle, and so may appear more or less homogeneous (figs. 19, 28, 34).

The presence of chromatin bars or rodlets at short intervals along the spirochaete in a stained preparation (figs. 24, 29, 32) gives rise to a so-called alveolar or chambered appearance, which has been much emphasised by Gross (1910) and those who have followed him. There is little or nothing new in Gross's observations, except the terms used to express his interpretations. However, his views have been so dogmatically and even polemically asserted that work previous to that of Gross has tended to be overlooked, although it was performed in many cases with due regard to careful cytological technique. The accentuation of differences of interpretation is merely indicative of partisanship, and does not conduce to the progress of knowledge.

The ends of *S. bronchialis* are tapering. Observed separately they may appear to be pointed (figs. 26, 33) or somewhat rounded (fig. 7) in stained preparations. Sometimes one end appears rather more tapering than the other (figs. 31, 39), a feature that is explained by extending attenuation at a previous division. So-called flagella are also thus explicable. These variations in the character of the ends of *S. bronchialis* also occur in other spirochaetes, such as *S. balbianii*, and were described by me at some length in 1909.

## V. MORPHOLOGICAL VARIATION IN *SPIROCHAETA BRONCHIALIS*

*Spirochaeta bronchialis* exhibits considerable polymorphism, resulting from the processes of growth and division. This range of variation is responsible probably for the varying dimensions of the organism given by different workers. The difference of size

exhibited by *S. bronchialis* can only be realised by examination and measurement of a large number of specimens derived from a series of cases. Measurements of spirochaetes from one or two cases only may be very fallacious, since the organisms observed may be all practically at the same stage of development.

By measuring a moderately large number (300) of bronchial spirochaetes, the discrepancies in length may be explained; I find that the length ranges from  $5\ \mu$  to  $27\ \mu$ . The size of a number of them centres around  $15\ \mu$ , while many of the others are about  $8\ \mu$  long. Castellani and Chalmers (1913) in their 'Manual of Tropical Medicine,' state on p. 1283 that one form of *S. bronchialis* is from  $15\ \mu$  to  $39\ \mu$ , while on p. 402 the commonest form is said to be  $7\ \mu$  to  $15\ \mu$  long. Thus my measurements give a lower limit for both minimum and maximum dimensions as stated by them, while a number of the spirochaetes that I have measured are about  $15\ \mu$ .

Macfie (1915) states that his smallest form was  $6\ \mu$  long, his largest  $13\ \mu$ , and that the average length of his specimens was  $8\ \mu$  to  $9\ \mu$ . When examining my series of slides, I found one—taken from a chronic case on a particular day—on which the spirochaetes seemed remarkably uniform. A number was drawn with the aid of a camera lucida and measured (figs. 7, 12, see also 13). It was found that almost everyone of them was from  $8\ \mu$  to  $9\ \mu$  long. Their measurements coincided almost exactly with those given by Macfie, and had this slide been the only one, erroneous conclusions would have resulted. Measurements of the spirochaetes from the same case on the days preceding and on subsequent dates showed that the range of length of *S. bronchialis* was considerably greater than was shown on that one occasion. As Macfie's dimensions were derived from two cases only, it was likely that the majority of the spirochaetes were at the same stage of development, and thus had attained about the same size. Many bronchial spirochaetes from Khartoum were either from  $14\ \mu$  to  $16\ \mu$  long, or  $7\ \mu$  to  $9\ \mu$  long. Definite evidence of transverse division in members of the former group has been obtained, and it is very likely that Macfie's forms measuring  $8\ \mu$  to  $9\ \mu$  so originated. Chamberlain (1911) having found bronchial spirochaetes in two typhoid patients in the Philippines, states that their average length is  $15\ \mu$ . Some of the spirochaetes that I examined in the Sudan measured about

15  $\mu$  long, but much depended on the age of the parasites and on the condition of the patient. For instance, the predominant type of *S. bronchialis* found in several chronic cases was an organism with two tapering ends, one sometimes slightly more rounded than the other, with a length of 13  $\mu$  to 16  $\mu$  (figs. 34, 35).

Dr. Taylor kindly sent me some slides of *S. bronchialis* from Uganda. I have drawn and measured spirochaetes from each of the slides he sent me, and can state that not only can I recognise the four types mentioned by him, but that the dimensions which I have obtained from his sketches agree in the main with mine (figs. 40-45). The range of length obtained from my measurements is from 5.5  $\mu$  to 19.5  $\mu$ . Dr. Taylor, in his Report for 1913, differentiated four groups of spirochaetes, which I have determined from his figures to measure from 6  $\mu$  to 9  $\mu$ , 6  $\mu$  to 8  $\mu$ , 15  $\mu$  to 18  $\mu$  and 9  $\mu$  to 11  $\mu$ , respectively. The first three were considered by him to be bronchial spirochaetes, the last occurred in the mouths of healthy persons. While the spirochaetes were thus grouped by Dr. Taylor, he recognised the existence of possible transitional forms and that one group gradually merged into another.

The length of *S. bronchialis* is often a factor of its age. Forms recently developed by the elongation of granules or coccoid bodies, naturally, are short (fig. 1-6). Small forms also arise by transverse division of older, longer ones. The nutrient medium in which the spirochaetes occur also seems to react upon their morphology. Organisms surrounded by thick stringy mucus may become stouter than those in more fluid sputum, which offers less resistance to their passage through it. Spirochaetes found in a denser medium tend to be both shorter and broader than those in a more fluid one in many cases, though no general statement can be made regarding this factor. The degree of relaxation obtaining in the organism when it was killed is also a factor in producing differences in the appearance of the coils of the organism, and consequently in its dimensions.

Polymorphism or morphological variation of *S. bronchialis* occurs not only with respect to length, but also in connection with breadth and the character of the ends of the organism. The breadth of the spirochaetes shows some variation, but it is less marked than the variations in length. Occasionally a spirochaete broader than the average is found (fig. 38). The breadth is not easily determined, but varies from 0.2  $\mu$  to 0.6  $\mu$ .

The ends of the spirochaetes show considerable variation. The degree of tapering manifested by them depends in part on the motion of the parent organism during division, as mentioned previously. Spirochaetes in which both ends taper, but wherein one is somewhat more pointed than the other, are fairly common (figs. 27, 34, 37, 39, 46). Others show the ends equally pointed (figs. 8, 10, 22, 26); yet others have relatively rounded ends after some tapering of the body has occurred (figs. 7, 31, 47, 48). Extreme tapering of one or both ends of the spirochaete have led to the erroneous interpretation of flagella in the case of other spirochaetes, such as *S. dentium*. Occasionally such an interpretation might be made in specimens of *S. bronchialis*, but examination of many of the organisms, both living and in stained preparations, confirms the fact that true flagella do not occur in the organism.

A membrane or 'crista' is present in *S. bronchialis* (figs. 10, 16), but is not always easy of detection. This difficulty of demonstrating the membrane would, perhaps, cause some workers to assign the organism to the genus *Treponema*, in which hitherto a membrane has not been satisfactorily demonstrated. Such classification, however, would be fallacious, inasmuch as the coils of a *Treponema* are said to be pre-formed while those of a spirochaete are not, but are capable of variation according to the rapidity or slowness of motion and to the density of the medium traversed.

#### VI. THE GRANULE PHASE OF *SPIROCHAETA BRONCHIALIS*

The formation of coccoid bodies or granules in *Spirochaeta bronchialis* has been studied in fresh preparations, using both ordinary illumination and the paraboloid condenser, as well as in stained preparations. As the organisms are small, there is sometimes some slight difficulty in studying the process of formation of these minute reproductive bodies, though it is relatively easy to observe in larger, somewhat stouter specimens about 11  $\mu$  to 16  $\mu$  long and about 0.3  $\mu$  broad examined at the right stage of development. Further, there seems to be a periodicity in the formation of granules in *S. bronchialis*, but the exact period has not yet been determined.

The process of formation as observed in life is as follows:—The cytoplasm at first is very finely granular, in fact, almost homogeneous. The chromatin bars appear as minute refractile

masses. A concentration of some of the cytoplasm occurs around each chromatin rodlet. These small concentrations gradually become oval, the outer cytoplasmic layer differentiates as a thin coat, and ultimately a series of coccoid bodies or granules is formed (figs. 46-49), lying usually transversely or slightly obliquely within the periplast sheath. Sometimes the coccoid bodies are set at liberty by a rupture appearing at one end of the spirochaete (fig. 50), at other times several ruptures, or disintegration of the sheath can be observed. A few empty sheaths have been found both in fresh and in stained preparations. Stained specimens show a series of darker lozenge-like coccoid bodies alternating with relatively clear, pale staining areas (figs. 47-49).

The formation of coccoid bodies takes place both in free spirochaetes and in those which have penetrated the delicate cells lining the air passages (fig. 52). Sometimes two or more spirochaetes have been found within a mononuclear cell. They may be in the ordinary trophic phase, or may be in process of formation of coccoid bodies. When within a cell the coccoid bodies often seem to be liberated by the disintegration of the periplast. Groups of coccoid bodies still retaining the outline of the spirochaete from which they originated (fig. 52) are of fairly frequent occurrence. When the coccoid bodies are released by a terminal rupture of the parent, they tend to form irregular clumps. The elongation of the granules and the emergence of very small spirochaetes from the groups of granules (fig. 51) have been observed in life. It is very probable that there is a definite period in the life of a spirochaete at which there is a marked differentiation of coccoid bodies. It must also be borne in mind that coccoid bodies may be present when spirochaetes as such cannot be detected.

In staining reactions, size and morphology, the coccoid bodies are different from any bacteria which may be present in the surrounding medium. They stain less darkly than cocci, and are smaller (fig. 52). Spirochaetal coccoid bodies have no capsule such as occurs commonly in bacteria.



## VII. SOME FURTHER REMARKS ON THE GRANULE STAGE OF SPIROCHAETES

In continuance of the remarks which I made on the subject of the granule stage of spirochaetes in these *Annals* (1914), Vol. VIII, pp. 471-484, I should like to draw attention to the following further evidence.

1. Noguchi, in his address before the Royal Society of Medicine, London, on October 20th, 1913, stated that he 'was able to demonstrate . . . granules in the pure cultures of *Treponema pallidum*. This phenomenon, however insignificant it may appear in itself, was destined to furnish a key to one of the most disputed problems of the past fifty years—namely, the problem of so-called parasyphilis, since it was this very idea that prompted me to undertake to search for *Treponema pallidum* in one form or another in the brains of general paralytics and in the spinal cord from cases of tabes dorsalis.' And again, 'I was led by the observation that *Treponema pallidum* sometimes assumes a granular form in cultures to re-study sections of paretic brains stained for the *pallidum*.'

2. Another interesting remark regarding the granule stage of spirochaetes may be found in Sir Patrick Manson's well-known book on Tropical Diseases. In the last (fifth) edition, published in 1914, on page 225, regarding the etiology of relapsing fever, he writes: 'Obermeier and von Jaksch describe certain refractile bodies present in the blood during the fever intermissions. The latter author says that he has observed the development of these bodies into short rods, from which the typical spirochaetes are eventually evolved.' These remarks have also appeared in earlier editions of the book.

3. Dutton and Todd, writing in 1905 concerning some of their experiments on the nature of human tick fever in the Congo Free State, record that: 'In some preparations of stomach or malpighian tubules [of spirochaete-infected ticks] no parasites were at first seen; but if a little human serum, taken from one who had never had tick fever, were added, in from 8 to 24 hours the preparations became fairly crowded with spirochaetes.' These observations may be explained by spirochaetes passing through a granule phase.

## VIII. THE SPIROCHAETES OF THE HUMAN MOUTH

Two species of spirochaetes were recorded as occurring in the human mouth about forty years ago. These are *S. buccalis*, Steinberg (often ascribed to Cohn, 1875), and *S. dentium*, Miller (often attributed to Koch, 1877). The former is said to be longer and thicker than the latter. The smaller spirochaete is also the more flexible. According to Hartmann and Mühlens (1906), *S. dentium* in culture measures  $4\ \mu$  to  $12\ \mu$  in length by  $0.3\ \mu$  to  $0.6\ \mu$  in breadth, or less if stains other than Löffler's are used, while *S. buccalis* is  $12\ \mu$  to  $20\ \mu$  in length by  $0.5\ \mu$  to  $1\ \mu$  in breadth. Hoffmann and Prowazek (1906) published microphotographs of specimens of *S. dentium* about  $5\ \mu$  to  $7.5\ \mu$  long, and of *S. buccalis* about  $8.5\ \mu$  to  $14.5\ \mu$  long. Intermediate types also occur, as was shown by these authors, such forms being named *Treponema intermedium* by Dobell (1912). Hartmann and Mühlens did not see much internal structure in *S. dentium*, but in *S. buccalis* they record the presence of an 'undulating' membrane in some specimens. Mühlens (1907) figured stained specimens of *S. buccalis* and *S. dentium* in which chromatin coloured granules were distributed along the bodies of the organisms. His figures of *S. dentium*, as measured by me, are about  $4\ \mu$  to  $7\ \mu$  long and of *S. buccalis* about  $15\ \mu$  to  $23\ \mu$ .

Noguchi (1912) has succeeded in cultivating a number of species of spirochaetes from the human mouth. He places these in the genus *Treponema*, making three new species, namely, *T. macrodentium*, *T. microdentium* and *T. mucosum*, but they cannot be easily distinguished morphologically. The last-named was obtained from cases of pyorrhea alveolaris. They all differ from *S. buccalis*.

In the course of my researches I have observed the parasites ascribed to Cohn and to Koch, these being the two common spirochaetes seen in the mouths of natives of the Sudan and of Europeans in England, as well as the forms described and cultivated by later investigators. Some of the mouth spirochaetes are not very active, but there is marked corkscrew and boring movement, and they are flexible. Tangles or tomenta of these mouth spirochaetes are common. Internal structure is seen with some difficulty, but in some specimens it can be determined, and chromatin granules are then observed.

*S. dentium* (figs. 53-57) has tapering ends, and varies in length from  $4\ \mu$  to  $10\ \mu$ , as found by measuring 40 specimens from some of my own preparations. *S. dentium* is so small that few details of its structure can be determined, and in consequence, it might be placed by some authorities in the genus *Treponema*. *S. buccalis* (figs. 58-68) has somewhat rounded or bluntly acuminate ends, and varies in length from  $9\ \mu$  to  $22\ \mu$ , as determined from 110 specimens measured. A slight membrane or crest may sometimes be observed in the latter species (figs. 60, 65). Intermediate forms were found, which might be considered to connect the two species.

The spirochaetes of the mouth take up stains well and with relative ease. Intracellular stages, if they occur at all, are uncommon. Multiplication by binary fission has also been observed. Coccoid bodies or granule stages of the mouth spirochaetes are formed, but appear to be relatively few in number.

No marked morphological difference has been observed between the spirochaetes occurring in the mouths of Europeans in England (four cases examined), and in natives of the Sudan (eight cases examined). However, it may be of interest to note that in the Sudan the predominant spirochaete in the mouths of the natives whom I examined was *S. buccalis*, *S. dentium* being relatively uncommon.

Spirochaetes that were either *S. dentium* or closely allied thereto were found in the mucus from the pharyngeal and laryngeal regions of a normal monkey in the Sudan.

A spirochaete of the human throat, often associated with fusiform bacilli, may be mentioned here. *Spirochaeta vincenti* occurs in the throat in certain conditions, such as Vincent's angina. It has recently been described by Drs. J. G. and D. Thomson (1914). It is said to be an elongate spirochaete, with a flexible, irregularly coiled body, tapering at both ends. The spirochaete, according to the present state of our knowledge, does not seem to be identical with *S. bronchialis*, differing from it morphologically and in the situations in which it is found. *S. vincenti* was named by Blanchard in 1906, but as no description of the spirochaete was given at the time of naming, the species name is probably not valid. Little is known of the range of morphological variation of *S. vincenti*. I have measured those drawn by Thomson and find that they are from  $10\ \mu$  to  $18\ \mu$  long, while the few figured by Mühlens (1907) I find to measure

9  $\mu$ , 10  $\mu$  and 23  $\mu$ . Castellani and Chalmers state that it is 12  $\mu$  to 25  $\mu$  long. It is possible that this species will have to be merged into another one, such as *S. bronchialis*, when it has been more fully investigated.

#### IX. THE SPIROCHAETES OF THE HUMAN MOUTH CONTRASTED WITH *S. BRONCHIALIS*

There are general differences between these two groups of spirochaetes, and these differences may be set forth with a view to aiding those workers in the tropics who may meet with cases of bronchial spirochaetosis. However, the said differences must not be emphasised too much or asserted dogmatically, as further work may lead to modifications.

The bronchial spirochaetes appear to be more active than those of the mouth.

*S. bronchialis* dies very rapidly outside the respiratory tract. This has also been noticed by Chalmers and O'Farrell and by Taylor. Oral spirochaetes, on the other hand, can live for some hours outside the human mouth.

Bronchial spirochaetes, as a rule, stain with difficulty and not so easily as those obtained from the mouth. The former are much more fragile and slender than *S. buccalis*.

Coccoid bodies are frequently produced by *S. bronchialis*, and are probably the cross-infective stage of the parasite. Coccoid bodies appear to be less frequently formed by oral spirochaetes.

Intracellular stages of *S. bronchialis* are occasionally seen. No such stages have been observed in the cases of spirochaetes from the mouth, which I have examined.

Tangles are not usually formed by *S. bronchialis*, but are commonly produced by numbers of spirochaetes aggregating in clusters in the oral cavity.

#### X. MODE OF INFECTION

The situation in which *Spirochaeta bronchialis* is found largely precludes its dissemination direct from man to man by the aid of insects, though they may serve as indirect transmitters of the parasite.

It is highly probable that man himself acts as the reservoir of the virus, and that direct contact of infected with healthy persons is responsible for the spread of bronchial spirochaetosis. The spray exhaled with the expired air appears to be contaminated with the resistant coccoid bodies produced by *S. bronchialis*. Such fine spray inhaled by an uninfected person whose bodily resistance is somewhat lowered—as after a chill—results in an attack of bronchial spirochaetosis. It is possible that spirochaetes as such might be exhaled in the spray, but as has been pointed out they are fragile and quickly die outside the body. It is also possible that the inhalation of dust contaminated with dry sputum from patients suffering from bronchial spirochaetosis may excite an attack, while the nasal secretions passed on to linen and allowed to dry, the linen then being packed indiscriminately with other soiled clothing, may serve to transmit the parasite to new hosts.

The agency of insects in spreading bronchial spirochaetosis is somewhat remote, but it must be remembered that certain flies are partial to sputum, which they not only ingest but which may soil their bodies and feet. While the frail, motile spirochaetes are not likely to be carried as such in or on the bodies of flies, it is quite possible that the more resistant coccoid bodies may be carried about by flies, and deposited by them either directly on the lips or nose of man, or in foods such as milk, whence they may reach the pharynx and perhaps pass downwards into the trachea and bronchi.

However, the direct contamination of healthy persons by infected ones is at present much the most likely means of dissemination of *S. bronchialis*.

## XI. GENERAL REMARKS

Certain points of interest connected with *Spirochaeta bronchialis* and bronchial spirochaetosis may now be considered.

That *Spirochaeta bronchialis* is disease producing is undoubted. The organisms have been found in cases of chest complaints, especially those with bronchitic symptoms, where no other cause for the affection was assignable. *S. bronchialis* was frequently the only organism present in the cases in the Sudan examined by me. Examination of a series of preparations from several chronic cases,

each considered separately, has shown that, on most of the occasions on which sputum was examined, bacteria of any kind were either few or absent. Sometimes organisms identical morphologically with *Diplococcus* (*Micrococcus*) *catarrhalis* were found on a single preparation of a series extending over a number of days, but this was uncommon. Occasionally bacteria were found in smears of mouth spirochaetes. On still rarer occasions cells from the lungs were observed in which endocellular spirochaetes and coccoid bodies related thereto were present in company with diplococci (fig. 52). The contrast between the coccoid bodies and the bacteria, both in size, morphology and staining reactions, was always noticeable. In one case only were pneumococci found associated with *Spirochaeta bronchialis*, the patient being a European soldier who might have become infected with spirochaetes after a chill had favoured pneumonic symptoms. No mycological agent has been found in any of my preparations. Chalmers and O'Farrell also have had a similar experience, and they state that the fungi responsible for bronchomoniliasis, broncho-nocardiasis, broncho-aspergillosis, bronchopenicilliosis, broncho-mucor-mycosis, broncho-sporotrichosis, have not been found by them 'in the sputum of their cases of bronchial spirochaetosis either by direct examination or by cultivation.' Further, Chalmers and O'Farrell succeeded in experimentally inducing bronchial spirochaetosis in a monkey, the parasites and symptoms closely resembling those in man (cf. figs. 19, 49).

It is also of interest to note that Chalmers and O'Farrell, and Taylor, both writing in 1913, arrived at the same conclusions regarding bronchial spirochaetosis in Khartoum and Uganda respectively. In each district *S. bronchialis* is held to be the cause of the haemorrhagic bronchitis noticed. Also each of the above workers state that there is an abundance of *S. bronchialis* during the attack, that the decrease in numbers synchronises with the abatement of symptoms, and that the spirochaetes disappear from the sputum entirely or are found with great difficulty after a few days. All these facts have a distinct significance when considering the etiology of the disease.

*S. bronchialis* I find to be morphologically different from the spirochaetes of the oral cavity, as mentioned previously. It is an entity in itself, and the malady caused by it also appears to be

distinctive. Its pathogenic action also seems to be unlike that of the debatable *S. vincenti* of Vincent's angina, in which a throat membrane may at times be produced.

*S. bronchialis* has a somewhat extensive though scattered distribution. Bronchial spirochaetosis has been reported from Europe, the cases being discussed by Chalmers and O'Farrell. It has also been recorded from Ceylon, India, the Philippines, and the West Indies. In East Africa the disease has been reported from the Anglo-Egyptian Sudan and Uganda. In West Africa the disease has been recently notified from various parts of the Gold Coast Colony. *Spirochaeta bronchialis* has thus been reported from four continents and from different districts in each. This scattered but wide distribution of the parasite suggests that it may be a very general but easily overlooked organism, of far more frequent occurrence than is usually supposed.

For the benefit of medical men who are interested in the subject and to whom Chalmers and O'Farrell's paper is not available, the treatment recommended by them is here quoted :—

'The first essential is rest in bed, good food, and ventilation. The second is arsenic in some form, preferably associated with glycerophosphates. These may be given by the mouth with excellent results, or intramuscularly as an injection of :—

Sodium cinnamate	...	...	...	0·05 grm.
Sodium cacodylate	...	...	...	0·10 „
Sodium glycerophosphate	...	...	...	0·10 „

Taylor, in Uganda, prescribes 'Arsenious acid by the mouth in increasing doses.'

At the time when these treatments were recommended (end of 1913) they had been employed in relatively few cases, but had given satisfactory results.

Chalmers and O'Farrell, and Taylor also discuss the co-existence of bronchial spirochaetosis with other chest diseases, such as lobar pneumonia, details of which should be sought in the original papers. Bronchial spirochaetosis may be suspected in atypical cases of pneumonia and bronchitis, and may be mistaken for incipient phthisis.

## XII. SUMMARY AND CONCLUSIONS

1. *Spirochaeta bronchialis* is an organism presenting marked polymorphism, a feature that has only been determined by the examination of numerous preparations from various patients. It produces bronchial affections in the Sudan and in other parts of the world.

2. *S. bronchialis*, as investigated in the Anglo-Egyptian Sudan, varies in length from 5  $\mu$  to 27  $\mu$ , and its breadth is about 0.2  $\mu$  to 0.6  $\mu$ . These variations are due to the processes of growth and division. Many of the parasites measure either 14  $\mu$  to 16  $\mu$  long, or 7  $\mu$  to 9  $\mu$ , the latter resulting from transverse division of the former. The ends show much variation in form, but approach the acuminate type on the whole. The discrepancies in dimensions given by the very few previous workers on *S. bronchialis* are the result of the measurement of a limited number of parasites. All such sizes can be found on some occasion during the progress of the disease, when a larger number of spirochaetes is examined.

3. The movements of *S. bronchialis* are active, but of relatively short duration, when it is removed from the body. The number of coils of the spirochaetes is rather an index of its rapidity of motion than a fixed characteristic of the species.

4. The motile phase of *S. bronchialis* is succeeded by one of granule formation, the granules or coccoid bodies serving as a resting stage from which new spirochaetes are produced. The formation of coccoid bodies and the reproduction of spirochaetes from them can be observed in life.

5. *S. bronchialis* is a species distinct from the spirochaetes occurring in the mouth. It differs from them in morphology, pathogenicity and in staining reactions. It is not a developmental form of any bacterium, and is an entity in itself.

6. The passage from man to man is effected most probably by means of spirochaetes, and especially coccoid bodies, that leave the body in the spray with expired air and by way of the nasal secretions. Owing to the fragility and short life of *S. bronchialis* extracorporeally, the resistant coccoid bodies in air, dried sputum and dust, and possibly also on the bodies of flies and other insects, are probably instrumental in inducing attacks of bronchial spiro-



chaetosis in human beings, especially those having a lowered bodily resistance, such as after a chill.

7. Spirochaetes of the type of *S. dentium*, measuring  $4\ \mu$  to  $10\ \mu$  in length, have been found in mouths of natives of the Sudan and in those of English people in England. Also *S. buccalis*, measuring  $9\ \mu$  to  $22\ \mu$ , has been obtained from the same sources.

8. *S. bronchialis* will probably prove to be of more frequent occurrence than is known at present.

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## EXPLANATION OF PLATE XXXIV

All figures were outlined with an Abbé-Zeiss camera lucida, using a Zeiss 2 mm. apochromatic objective and compensating ocular 18. The magnification is approximately 2,600 diameters.

Figs. 1-39. *Spirochaeta bronchialis* from the Anglo-Egyptian Sudan. Trophic stage.

Figs. 1-6. Small, young forms of *S. bronchialis*.

Figs. 1, 3. Spirochaetes with both ends slightly differing in form.

Figs. 2, 4. Forms with both ends alike. In fig. 2 the edge of the membrane shows as a line on the body.

Figs. 6-13. *S. bronchialis*. Growth forms showing variation in thickness and length.

Fig. 10. Spirochaete with relaxed membrane.

Fig. 13. Spirochaete drawn from preparation obtained from Kodok, Sudan.

Figs. 14-24. Longer forms.

Fig. 16. Form with slight membrane.

Figs. 19, 23. Forms with many small coils, fixed when in rapid motion.

Fig. 19 drawn from specimen from an experimentally infected monkey.

Figs. 14, 20. *S. bronchialis* from a case at Kodok.

Fig. 22. The edge of the membrane (crista) shows as a wavy line on the body.

Figs. 25-35. Some of the commoner ('average') types of *S. bronchialis*, showing variation in the number of coils.

Figs. 36-39. Some of the largest forms of *S. bronchialis* encountered.

Fig. 36. Spirochaete with slight membrane.

Fig. 37. Form with one end more pointed than the other.

Fig. 38. Very broad form.

Fig. 39. Long form with slight membrane.

Figs. 40-45. *Spirochaeta bronchialis* from Uganda.

Figs. 40-44. Trophic forms showing the same appearances and structure as the average types of *S. bronchialis* occurring in the Sudan.

Fig. 45. Parasite showing the formation of coccoid bodies.

Figs. 46-52. The granule phase of *S. bronchialis* from cases in the Sudan.

Figs. 46-49. Free spirochaetes containing coccoid bodies. Fig. 49 drawn from a preparation obtained from an experimentally infected monkey.

Fig. 50. Coccoid bodies in process of liberation at one pole of the spirochaete.

Fig. 51. Cluster of small coccoid bodies with young spirochaetes emerging from the group.

Fig. 52. Part of cell from the air passages, containing two spirochaetes, a group of coccoid bodies retaining the arrangement of the spirochaete from which they were liberated, and two diplococci.

Figs. 53-57. *Spirochaeta dentium* from mouths of Europeans and natives of the Sudan.

Figs. 53, 54. *S. dentium*, from natives of the Sudan.

Figs. 55, 56. *S. dentium*, from Case 1, Cambridge.

Fig. 57. *S. dentium*, from Case 2, Cambridge.

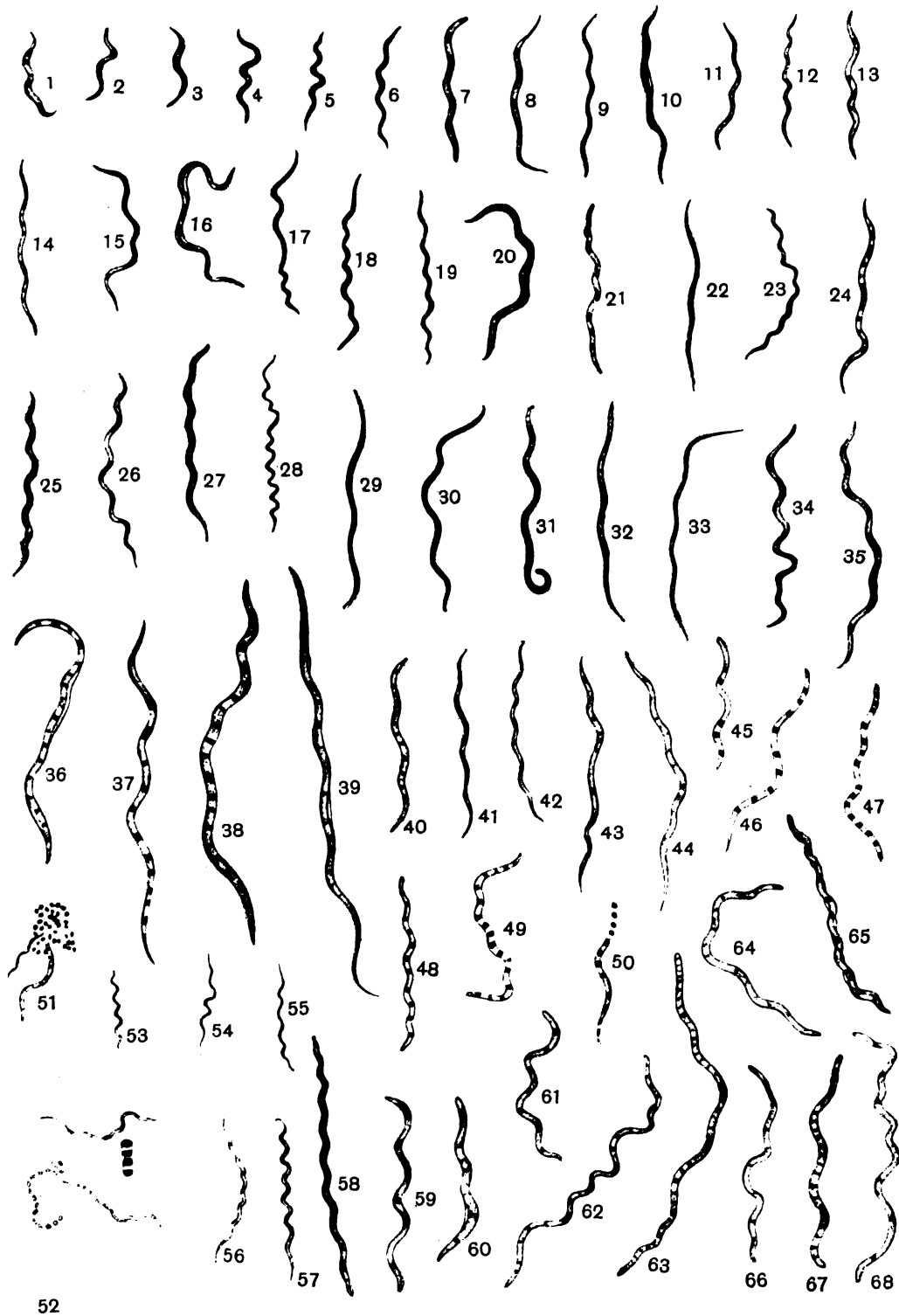
Figs. 58-68. *Spirochaeta buccalis* from mouths of Europeans and natives of the Sudan.

Figs. 58, 62, 63, 65. *S. buccalis*, from Case 1, Cambridge. Fig. 65 shows a membrane or crista.

Fig. 59. *S. buccalis*, from Case 2, Cambridge.

Fig. 64. *S. buccalis*, from Case 1, Liverpool.

Figs. 60, 61, 66-68. *S. buccalis*, from natives of the Sudan. In fig. 60 a slight membrane is indicated.



H. B. F. del.

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*SPIROCHAETA BRONCHIALIS*, Figs. 1—52  
*SPIROCHAETES* FROM HUMAN MOUTH, Figs. 53—68



# NOTES ON CERTAIN ANIMAL PARASITES OF DOMESTIC STOCK IN SIERRA LEONE

[*Being the Fourth Report of the Thirty-second Expedition of the  
Liverpool School of Tropical Medicine, 1914-15.*]

BY  
WARRINGTON YORKE  
AND  
B. BLACKLOCK

(*Received for publication 29 June, 1915*)

## SERIOUS EPIDEMICS OF DISEASE AMONGST CATTLE IN SIERRA LEONE

From time to time heavy losses have been experienced by cattle owners in Sierra Leone owing to epidemics, during which large numbers of cattle die.

In December, 1914, we were requested by the Administration to investigate the nature of a serious epidemic which had broken out amongst cattle in the northern part of the country. The cattle in question had been brought down originally from French Senegal; they were practically all bullocks, owing to restrictions respecting the export of cows put into force by the French Administration.

We proceeded to Batkanu, the capital of the Karene district, but were informed on our arrival that the epidemic had ceased and that the animals were no longer dying. We found 90 cattle in the Government *Warri*, all of which appeared to be in a healthy condition. Most of the animals were in the Karene district during the epidemic, and had remained apparently uninfected; a few, however, had been ill, and seemed to have recovered. Whether

these animals had really suffered from the infection which had resulted in the death of large numbers of others is uncertain, but Dr. Clearkin, the medical officer in charge of the district, was of the opinion that this was the case. We were informed that the epidemics occurred towards the end of the rainy season, and that they coincided with the appearance of large numbers of a fly (*Stomoxys nigra*), which was held responsible. This hypothesis is in accordance with the history given by the natives; those questioned by us stated that the disease appeared every five or six years, during the rainy season, and that it was due to the bites of the fly (*Stomoxys*). It was believed that the infection was contracted by the animals whilst being brought down from French country, where cattle breeding is carried on extensively.

The course of the disease was rapid, the symptoms being fever, staring coat, emaciation, and death in a month or six weeks.

Fresh and stained preparations of blood of each of the 90 cattle in the *Warri* were examined, and rats were inoculated with blood from every tenth animal. As a result of this examination four cattle were found to be infected with trypanosomes; two with *T. vivax*, one with *T. congolense* and one with *T. gambiense*. In two of the animals *Piroplasma bigeminum* was found, and in about 30 per cent. a parasite belonging to the genus *Theileria*. Two healthy oxen were inoculated with blood from each of the animals suffering from infection with *T. vivax*. The animals inoculated from the first ox remained healthy, and trypanosomes were never seen in their blood; those inoculated from the second, which also harboured *Piroplasma bigeminum* in its blood, became infected both with *T. vivax* and with *Piroplasma bigeminum*, but exhibited no signs of disease, and were alive and in good condition when we left the colony three months later.

In view of the fact that we did not see these animals until the epidemic had ceased, it was impossible for us to make any post-mortem examination, and there was no material at our disposal upon which a definite conclusion could be reached as to the cause of the great mortality which had occurred. Although the examination of blood proved that the cattle were infected with several protozoa, any one of which is potentially the cause of epidemics fatal to cattle, it would not be possible to make any definite statement on the



subject until observations can be made on the spot during the time when the epidemic is running its course, so that animals can be examined systematically, the clinical course of the disease watched and material obtained immediately after death. We cannot emphasise too much the importance of properly directed and sustained work in the investigation of problems of such economic importance; it is only by this means that the true nature of these ever-recurring epidemics will be elucidated.

### TRYPANOSOMIASIS

During our visit to Sierra Leone we had the opportunity of examining a considerable number of domestic stock, both in the Colony and in the Protectorate. In all, 143 cattle were examined, and trypanosomes were found in nineteen. The proportion of infected cattle must, without doubt, be very much greater than this, as in the majority of cases an examination of a single fresh preparation of the blood was all that could be undertaken. These cattle, which were brought to Freetown for slaughter, appeared to be the most heavily infected; trypanosomes were discovered in the blood of fourteen out of thirty-four animals immediately after they had been killed at the Military slaughter-house. A probable explanation of this is that so soon as animals begin to lose condition they are taken by the natives to be sold in the Freetown market. In the nineteen infected animals *T. congolense* was found eleven times, *T. vivax* twice, a double infection of *T. congolense* and *T. vivax* five times, and *T. gambiense* once.

The blood of ten dogs was examined, but in no cases were trypanosomes found. One of the animals was, however, proved to be infected with *T. congolense* by inoculation of rats. No instance of infection with trypanosomes was found in the seven goats and seven sheep examined.

In connection with the information derived from examination of domestic stock it is of interest to refer to observations on the amount of trypanosome infection obtaining in *G. palpalis*.

Four hundred wild *Glossina palpalis* caught on the Cape Lighthouse Peninsula were dissected and examined; trypanosomes were found in twenty-one of them.

TABLE I.—Showing the manner in which trypanosomes were recognised in infected domestic stock

No.	Animal	Trypanosomes found in peripheral blood	Animals inoculated	Result of inoculation	Diagnosis
1	Ox 11 ...	<i>T. congolense</i>	...	...	<i>T. congolense</i>
2	Ox 23 ...	<i>T. vivax</i>	Ox I Ox II Rat 15a Rat 15b Rat 15c	Negative Negative Negative Negative Negative	<i>T. vivax</i>
3	Ox 88 ...	<i>T. congolense</i>	Rat 21	<i>T. congolense</i>	<i>T. congolense</i>
4	Ox 89 ...	<i>T. vivax</i>	Ox III Ox IV Rat 16a Rat 16b Rat 16c	<i>T. vivax</i> <i>T. vivax</i> Negative Negative Negative	<i>T. vivax</i>
5	Ox 100 ...	Negative	Rat 24	<i>T. gambiense</i>	<i>T. gambiense</i>
6	Ox 108 ...	<i>T. congolense</i>	...	...	<i>T. congolense</i>
7	Ox 111 ...	<i>T. congolense</i>	...	...	<i>T. congolense</i>
8	Ox 112 ...	<i>T. congolense</i>	...	...	<i>T. congolense</i>
9	Ox 120 ...	<i>T. congolense</i>	...	...	<i>T. congolense</i>
10	Ox 121 ...	<i>T. congolense</i>	...	...	<i>T. congolense</i>
11	Ox 137 ...	{ <i>T. congolense</i> <i>T. vivax</i>	Rat 11a Rat 11b	Negative Negative	{ <i>T. congolense</i> <i>T. vivax</i>
12	Ox 140 ...	<i>T. congolense</i>	Rat 12a Rat 12b	<i>T. congolense</i> <i>T. congolense</i>	<i>T. congolense</i>
13	Ox 141 ...	<i>T. congolense</i>	Rat 13a	Negative	<i>T. congolense</i>
14	Ox 142 ...	<i>T. congolense</i>	Rat 13b	Negative	<i>T. congolense</i>
15	Ox 143 ...	<i>T. congolense</i>	...	...	<i>T. congolense</i>
16	Ox 144 ...	{ <i>T. congolense</i> <i>T. vivax</i>	... ...	... ...	{ <i>T. congolense</i> <i>T. vivax</i>
17	Ox 145 ...	{ <i>T. congolense</i> <i>T. vivax</i>	... ...	... ...	{ <i>T. congolense</i> <i>T. vivax</i>
18	Ox 146 ...	{ <i>T. congolense</i> <i>T. vivax</i>	... ..	... ...	{ <i>T. congolense</i> <i>T. vivax</i>
19	Ox 147 ...	{ <i>T. congolense</i> <i>T. vivax</i>	.. ...	... ...	{ <i>T. congolense</i> <i>T. vivax</i>
20	Dog 155 ...	Negative	Rat 30a Rat 30b	<i>T. congolense</i> <i>T. congolense</i>	<i>T. congolense</i>

TABLE 2.—Giving results of dissection of wild *Glossina palpalis* found to be infected with trypanosomes

No. of fly	Salivary glands	Proboscis	Intestine	Result of inoculation
1	o	o	+++	2 rats, negative
2	o	++	o	
3	o	++	++	1 rat, gut and proboscis contents, negative
4	o	+++	o	
5	o	++	o	
6	o	++	o	
7	o	+++	o	
8	o	+	o	
9	o	+++	+++	{ 1 rat, gut contents, negative 1 rat, proboscis contents, died on 6th day
10	o	+	o	
11	o	+++	o	
12	o	+++	o	
13	o	+	o	
14	o	++	o	
15	o	o	+	
16	o	+++	+++	{ 1 rat, gut contents, died on 6th day 1 rat, proboscis contents, died on 6th day
17	o	+	o	
18	o	+	o	
19	o	+	o	
20	o	++	+++	{ 1 rat, gut contents, negative 1 rat, proboscis contents, negative
21	o	+	o	

+ Signifies trypanosomes scanty.

++ Signifies trypanosomes numerous.

+++ Signifies trypanosomes swarming.

It will be seen from Table 2 that of the twenty-one flies found to contain trypanosomes, the proboscis alone was involved in fifteen, the gut alone in two, whilst there was a heavy infection of both gut and proboscis in four. No instance of invasion of the salivary glands was encountered. Assuming that these parasites were trypanosomes pathogenic to man and domestic stock, the result of previous work allows us to conclude that fifteen of the above flies were infected with *T. vivax*, four with *T. congolense*, and the remaining two with a trypanosome belonging either to the Congolense or the Gambiense group; which, we are unable to decide owing to the fact that the cycle of development of the trypanosome in the fly was incomplete. Inoculations into rats were made, with negative results in each case, from five of the flies found to be infected. Unfortunately in two instances (Flies 9 and 16) the rats which had received the contents of the proboscis died six days after inoculation, but in two other cases (Flies 3 and 20) the rats lived long enough to have become infected. The fact that these animals did not become infected does not prove that the trypanosome found in the flies was not *T. congolense*, as it has now been established that it is always easy to infect rats with this trypanosome. A similar failure of *T. congolense* to establish itself in these animals is seen in the case of three rats inoculated respectively from Oxen 137, 141 and 142 (see Table I), which were found on direct examination of the blood to be suffering from an infection with *T. congolense*. Previously the trypanosome in question would have been designated *T. nanum*, but recent work shows that the difference between the latter parasite and *T. congolense* is only an apparent one.

A small number (95) of wild *G. palpalis* was fed on rats; no infection resulted.

#### BABESIASIS AND THEILERIASIS

Whilst examining blood films of cattle, species belonging to the genera *Babesia* and *Theileria* were seen. In about 5 per cent. of the animals examined a few large double pear-shaped bodies and an occasional large amoeboid form were encountered in the red cells. This parasite was undoubtedly *Piroplasma bigeminum*.

The other parasite occurred much more frequently, being found in between 20 and 30 per cent. of the animals examined. Small rod- and ring-shaped bodies showing distinct cytoplasm and chromatin were seen in the red cells. In some animals 1 or 2 per cent. of the cells were invaded, whilst in others only an occasional parasite was found. These bodies seem to be identical with those discovered by Macfie (1914) in cattle in Nigeria. They did not appear to produce any symptoms of disease. Most of the animals in which they were found were in perfect health; a few were emaciated, but in these trypanosomes were also found.

Owing to the fact that so large a proportion of cattle were discovered to harbour these bodies in their blood—usually they were present in very small numbers only—we did not consider that inoculation experiments into local cattle would afford any evidence as to the inoculability or otherwise of the parasite. No 'blue bodies' of Koch were found in the blood, or in smears made from the kidneys, liver, spleen, lungs, or bone marrow of six infected oxen. Theiler (1907) describes *Theileria parva* and *Theileria mutans* as being very similar in appearance. He differentiates between them on the following grounds. *Th. parva* is very virulent, is non-inoculable, and is characterised by the presence of the 'blue bodies' of Koch; whereas *Th. mutans* is non-virulent, is inoculable, and the 'blue bodies' of Koch are not found. There is little reason to doubt, therefore, that the parasite in question is *Theileria mutans*.

All the ticks which could be found were collected from each of the ninety cattle examined in the Warri at Batkanu; the animals were thrown and carefully searched. The only species encountered were *Boophilus australis* (126 adults, and 395 nymphs) and *Amblyomma variegatum* (4 adults, and 28 nymphs). We are indebted to Professor G. H. F. Nuttall for kindly determining them.

### CONCLUSIONS

1. Cattle are not bred to any extent in the Colony of Sierra Leone or in most parts of the Protectorate. Bullocks are, however, imported from French Senegal in considerable numbers, and gradually find their way down to Freetown for slaughter.

2. Serious outbreaks of disease in the form of epidemics occur amongst the cattle of Sierra Leone.

3. These epidemics have been attributed to various causes, but their real nature is still obscure.

4. Trypanosomiasis of cattle is common. Of the thirty-four animals examined at the slaughter-house in Freetown, fourteen (41 per cent.) were found to be infected. As only a single blood film was examined, the real percentage of infections is certainly much higher.

5. *T. congolense* and *T. vivax* are the parasites most commonly found. *T. gambiense* was met with once.

6. About 5 per cent. of the animals examined were found to be infected with *Piroplasma bigeminum*.

7. *Theileria mutans* was encountered in between 20 and 30 per cent. of the cattle examined.

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## THE ETIOLOGY OF JUXTA-ARTICULAR SUBCUTANEOUS NODULES

BY

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*(Received for publication 16 June, 1915)*

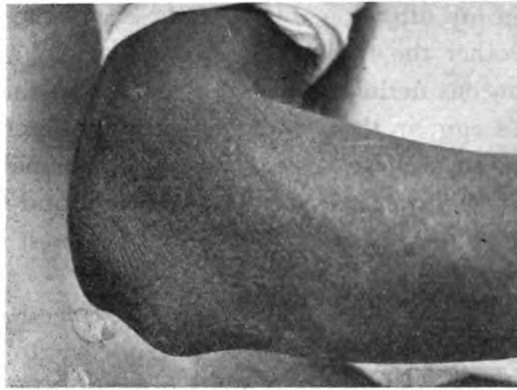
Whilst engaged, during the months of June, July and August, 1913, in examining the natives of the low-lying part of the Dedza district of Nyasaland for sleeping sickness, two facts soon forced themselves upon my attention. One of these was the prevalence of yaws and the other the frequent occurrence amongst these people of firm subcutaneous nodules, varying in size from that of a pea to that of a duck's egg, in the vicinity of joints. Observations were, therefore, made on a series of 2,378 adults (964 males and 1,414 females) and 567 children, natives of this district, and the results are presented in this paper, as they seem to be of interest in elucidating the etiology of such nodules.

The nodules which, as I have said, vary considerably in size, are firm, painless, not tender, show no tendency to break down, and are always situated in close relationship to the subcutaneous portions of bone. They are, however, freely movable on the bones and the skin over them, except in a few cases where definite signs of trauma accounted for adhesion to the skin. Their favourite site is the posterior border of the ulna, about two inches from the tip of the olecranon, and they were found, in this situation, on one or both sides, in 55 of the 2,378 adults examined. Other fairly common sites are the great trochanter of the femur and the lower part of the patella: less commonly they were found over the malleoli, and in one case on the zygoma. Frequently several were found in one individual at these various positions. At the patellar site they were usually accompanied by enlarged bursa. Altogether in 80, out of the 2,378 adults examined, they were found in one or more positions. They were never found in children, of whom 567 were examined for them.

These observations led me to the conclusion that the nodules are those referred to, in works on Tropical Medicine, as 'Juxta-articular Nodules,' and they have not, so far as I am aware, been reported from this part of Africa before.

Of the 964 men, 14 were actually suffering from, and 160 showed clear indications of having had, yaws: of the 1,414 women, 29 and 234 respectively: for the 567 children, the figures were 15 and 70.

With eight exceptions, the 80 subjects of these subcutaneous nodules had characteristic scars of, and admitted having had, yaws: of the eight exceptions, seven had the typical pigmented, finely wrinkled scars, but denied infection. Yaws is so commonly



Subcutaneous Juxta-articular Nodule on the forearm of a man who had yaws as a child.

contracted by these people in infancy and early childhood that many may forget all about it by the time they reach adult years. The opinion which I hold that these subcutaneous nodules are a late manifestation of yaws, comparable to, but without the tendency to softening of syphilitic gummata, is not, I think, invalidated by the above-mentioned exceptions. In most cases the nodules appear years after the manifest lesions of yaws have healed, but in four cases they were found in persons still showing unhealed granulomata.

For comparison, I was able subsequently to examine 327 adults



and 266 children in another (Mlanje) district of the Protectorate where yaws is not, so far as I am aware, known. The numbers are, unfortunately, small. No recent case was found amongst these people, who belong to quite a different tribe from the inhabitants of Dedza district, and many of whom have, within recent years, immigrated from Portuguese territory. Only two of the adults admitted having had yaws or showed indications of infection: both of these had characteristic yaws scars and also subcutaneous nodules: one had also beautifully pitted hands and feet—a condition to which Castellani (1910) has drawn attention. These two persons had contracted the disease in another part of the country. None of the 266 children examined showed any indications of the disease.

In the *Journal of the London School of Tropical Medicine* (1913), in a review of a paper by Ouzilleau, it is stated that in Mbomau adult *Filaria volvulus* were found 'in cysts or tumours, generally superficial, situated under the skin,' and 'the conclusion must be drawn that the volvulus cysts situated near joints do not differ markedly from those tumours known as "Juxta-articular Nodules."' 'Brumpt had observed many cases of these nodosities in Uganda and some on the Ouelle' . . . 'in every case of this sort seen by the author he had punctured the tumour and found the embryos and sometimes fragments of the adult of *Filaria volvulus*.' I therefore punctured one nodule, and excised two from the forearm in another case: neither in the small quantity of material removed by puncture, nor in scrapings from the cut surface of the excised nodules was anything resembling a filaria seen. Microscopically, fragments of fibrous connective tissue but no parasites or organisms of any sort were found. On section the nodules were solid, tough and lay embedded in subcutaneous fat.

Under the tertiary stage of yaws, Castellani and Chalmers mention the occurrence of gummatous-like nodules which soften and break down: such a condition I have observed in a few cases of yaws: the nodules to which I refer have no such tendency to softening.

The observations recorded above point strongly, in my opinion, to these nodules being a late manifestation of yaws, and they so closely resemble the 'Juxta-articular Nodules' described in

Castellani and Chalmers' *Manual of Tropical Medicine* that there can be little doubt of their identity with the latter.

Since these observations were made, I have read in the *Tropical Diseases Bulletin* (1913) that Mouchet and Dubois have observed similar nodules, and state that the natives in the Congo consider them a late manifestation of yaws. The sectional editor adds that Jeanselme's Juxta-articular Nodules are due to a fungus—*Nocardia carougeai*, Brumpt, 1910—but that in Africa, nodules induced by *Filaria volvulus* may closely simulate them.

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# ANKYLOSTOMIASIS IN DOGS IN SIERRA LEONE

[Being the Fifth Report of the Thirty-second Expedition of the  
Liverpool School of Tropical Medicine, 1914-1915.]

BY

WARRINGTON YORKE

AND

B. BLACKLOCK

(Received for publication 9 July, 1915)

Ankylostome infection appears to be universal in dogs in Freetown; of seven examined by us, all were found to be heavily infected. More detailed examination showed that the infection was due to two parasites—*Ankylostoma caninum*, Ercolani, 1859, and *Ankylostoma ceylanicum*, Looss, 1911—which were present in the intestines in about equal numbers. These species are readily distinguished one from the other by the characteristic arrangement of their teeth. The mouth of *Ankylostoma caninum* is armed with three pairs of prominent ventral teeth, whereas in *Ankylostoma ceylanicum* there is one pair of large ventral teeth, and one very small pair near the base of the former, but on a slightly deeper plane. These characters are illustrated in Figs. 1 and 2.

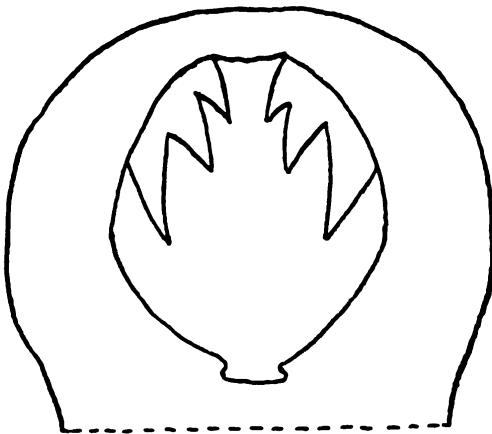


FIG. 1. Mouth of *Ankylostoma caninum*.  
x 300.

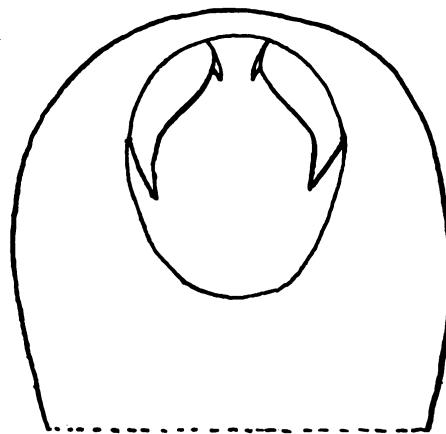


FIG. 2. Mouth of *Ankylostoma ceylanicum*.  
x 300.

The bursa of the males is very similar in the two species. It consists of two large lateral and a small dorsal lobe. The arrangement of the rays is as follows: In each lateral lobe there is an anterior ray which is cleft, an antero-external ray, a median ray which is doubled, and a postero-external ray which arises from a common trunk with the single posterior ray. In the dorsal lobe is the posterior ray, which exhibits slight differences in the two species. In both it is bifurcated in its terminal third, and each of the branches is at its extremity tridigitate. It is in the character of these terminal digitations that the slight difference is found (see figs. 3 and 4). In

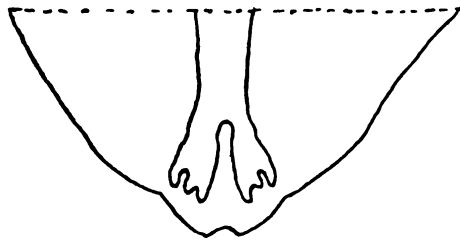


FIG. 3. Posterior ray of bursa of *Ankylostoma caninum*.  $\times 300$ .

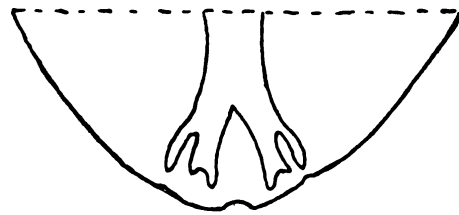


FIG. 4. Posterior ray of bursa of *Ankylostoma ceylanicum*.  $\times 300$ .

both species the two inner digitations are small, being separated by a mere notch. In *Ankylostoma caninum*, the cleft separating the two inner from the outer digits is shallow, but in *Ankylostoma ceylanicum*, the cleft is deep, being about half the length of the branch of the posterior ray.

The average length of *Ankylostoma caninum* is greater than that of *Ankylostoma ceylanicum*. A few were measured by us with the following results:

*Ankylostoma caninum*: Males 6.5-8 mm., females 7.5-14.5 mm.

*Ankylostoma ceylanicum*: Males 6 mm., females 7-10 mm.

The discovery of *Ankylostoma ceylanicum* in the dog in Sierra Leone is of interest. This species was first described by Looss in 1911, from a civet cat from Colombo. It was subsequently found by Lane (1913) in the dog and cat in Bengal, and also in a lion from the Calcutta Zoological Garden. In the same paper Lane described this parasite as occurring occasionally in human beings.

Gomes de Faria, in Brazil (1910), found dogs and cats infected with *Ankylostoma caninum* and a parasite which he described as a

new species under the name *Ankylostoma braziliense*, but Leiper (1913), from a comparison of figures published by Lane and de Faria, concludes that *Ankylostoma braziliense* and *Ankylostoma ceylanicum* are identical.

We had no opportunity of determining whether *Ankylostoma ceylanicum* occurs in human beings in Sierra Leone, where infection with Ankylostominae is exceedingly common. The importance of ascertaining whether this species occurs in man is obvious. If *Ankylostoma ceylanicum* is found in human beings in Freetown, the dog reservoir of the infection is a factor which must be borne in mind when prophylactic measures are under consideration.

### CONCLUSIONS

Dogs in Freetown are heavily infected with Ankylostominae.

The species found were *Ankylostoma caninum* and *Ankylostoma ceylanicum*; they were present in about equal numbers.

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## STUDIES IN BLACKWATER FEVER\*

IV.—NOTE ON A CASE OF QUARTAN  
MALARIA ASSOCIATED WITH  
BLACKWATER FEVER

BY

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*(Received for publication 2 July, 1915)*

## ONE CHART

*Past History*

X.Y.Z. European, W. Africa. Length of residence in Africa four tours.†

31.3.13.—6.4.13. Sunstroke.

29.4.13.—3.5.13. Sciatica and malaria. Has been ill with more or less fever for last seven days

*Present History*

12.8.13, 4 p.m. Admitted to hospital. Condition: Skin yellow, unhealthy looking; conjunctivae pale; gums pale; tongue dry yellow-brown fur. Liver palpable  $1\frac{1}{2}$ " below costa. Pulse, 108. Temperature,  $101\cdot8^{\circ}$ .

14.8.13. Patient progressing favourably until

15.8.13, at 4 p.m., feeling of distress, anorexia, frequent shivers, inclination to vomit. 10.30 p.m. Blackwater passed.

16-19.8.13. Frequent vomiting, extreme jaundice, increasing heart-failure.

18.8.13. Slight oozing of blood from gums.

19.8.13. Profound collapse. Death 1.20 p.m.

\* Part I: *Annals of Trop. Med. & Parasitol.*, 1913. Part II: *Ibid.*, 1914.  
Part III: *Ibid.*, 1915, p. 201.

† A tour is a year.

In the accompanying tables I have given an abstract of the available data as to the case. They are presented here for the sake of completeness rather than for any bearing they have on the problem raised.

*Record of Quinine*

12.8.13., 4 p.m. Quinine grs. vi. intramuscularly.

13.8.13. '*Mist. quin.*' quartis horis (at what hours given not stated).

14.8.13. Presumably '*Mist. quin.*' continued.

15.8.13. Presumably continued during the day, ? till 4 p.m., as it is stated that in the evening it was discontinued, but whether before or after the blackwater is not stated. Aspirin 10 grs. presumably at or after 4 p.m.

(NOTE.—The *Mist. quininae* of the B.P.C. contains gr. i. to 3i, but whether the above mixture is this, it is impossible to say.)

BLOOD

	Red cells	Hgb.	Total count leucocytes	Percentage count number counted	Large mono-nuclear	Lymphocytes	Poly-nuclear	Eosinophil	Parasites*
12.8.13 ...	...	...	...	...	...	...	...	...	Quartan parasites, scanty rings
13.8.13 ...	...	...	...	...	...	...	...	...	
14.8.13 ...	...	...	...	...	...	...	...	...	
15.8.13 ... (10.30 p.m.)	...	...	...	300	6	11	83	...	Parasites negative
16.8.13 ...	2,500,000	50%	(Tallqvist)	...	...	...	...	...	
17.8.13 ...	1,400,000	30%	...	...	...	...	...	...	
18.8.13 ...	600,000†	25%†	18,000	300	14	15	65	6	Parasites negative

\* Five Blood examinations were made, but the dates of two of them are not stated.

† NOTE.—These data imply a color index of 2.1 !



## URINE

Date	Time	Quantity	Reaction	Colour	Hgb.	Casts, etc.
13.8.13 .....	...	...	...	...	No alb.	Bile present
14.8.13 .....	...	...	...	...	...	
15.8.13 .....	Apparently no urine passed before 10.30 p.m.					
" .....	10.30 p.m.	220 c.c.	Faintly alkaline	Black	Met. Hgb.	Granular casts
16.8.13 .....	2 a.m.	70 c.c.	Faintly acid	...	"	"
" .....	3 a.m.	14 c.c.	Acid	Rather lighter	"	"
" .....	9 a.m.	145 c.c.	Alk.	...	"	"
" .....	9.40 a.m.	170 c.c.	(In the motion)	...	...	...
" .....	12.15 p.m.	240 c.c.	Alk.	...	Met. Hgb.	"
" .....	6 p.m.	240 c.c.	"	Much lighter	"	"
17.8.13 .....	12.30 p.m.	278 c.c.	Acid	Darker	"	No casts
" .....	—	230 c.c.	Alk.	"	"	"
18.8.13 .....	2 a.m.	360 c.c.	Acid	Clearing	"	"
" .....	11.45 a.m.	50 c.c.	"	Amber	"	"
" .....	10.30 p.m.	155 c.c.	"	Light amber	No alb.	Trace of bile
" .....	11 p.m.	20 c.c.	"	"	"	"
19.8.13 .....	12.45 a.m.	60 c.c.	"	"	"	"
" .....	12 noon	?	"	...	...	No casts

## POST-MORTEM (PARTIAL) FIVE HOURS AFTER DEATH

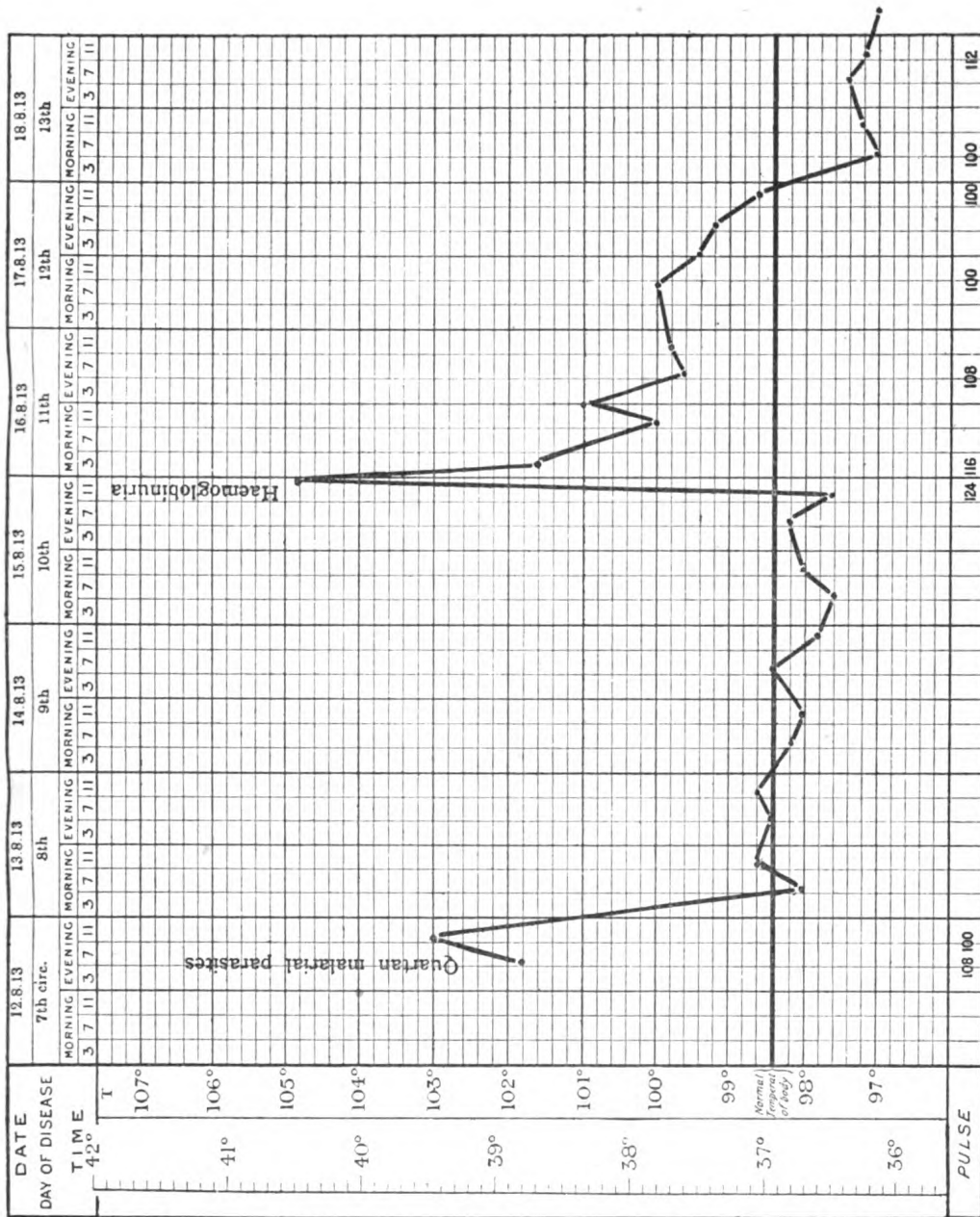
Skin ... ..	Very yellow	
Liver ... ..	Very large, yellow mottled, soft, old adhesions	Fatty degeneration, perilobular fibrosis. No parasites, some pigment
Spleen ... ..	Large	No parasites, some pigment
Stomach ... ..	Petechiae oozing blood, contents green bile	
Small intestine ... ..	Petechiae oozing blood	
Gall bladder ... ..	Yellow bile	
Kidneys ... ..	Large, pale	Many lobules blocked with 'haemoglobin' casts
Bone marrow ... ..	Pale	No parasites, some pigment

This case is of interest for the following reasons.

1. Immediately after the blackwater, 15.8.13, 10.30 p.m., no malaria parasites or pigment were found, i.e., there is no evidence of a malaria infection, yet on 12.8.13, 4 p.m., scanty quartan parasites were found, i.e., the patient at that time was infected with malaria. The records do not permit of any conclusion as to when evidence of malaria infection ceased, for although two other blood examinations (presumably negative) were made, yet it is not stated when they were made, i.e., whether before or after the blackwater. Further, the only evidence of a malaria infection post-mortem is the presence of pigment, parasites being absent. It is improbable, however, that a quartan infection was got rid of by three days' quinine treatment. We have then a case of blackwater fever, negative as regards malaria, subsequent to the blackwater, but positive three days previously, and also positive (pigment) post-mortem.

2. The diagnosis of quartan parasites on the 12th is supported by the character of the chart (see p. 433), which is quartan in character. If we assume that the rise on the 15th is a quartan paroxysm, then we have the actual paroxysm in this case, for some reason or other, associated with blackwater. It may be argued, however, that the paroxysm on the 15th is due to the onset of blackwater *per se*, and that it is a coincidence that it has occurred when we should have naturally expected a quartan paroxysm. This possibility cannot be disproved, but also the possibility—if nothing more—exists that this is not so, and that in this case a quartan paroxysm is closely concerned with the blackwater attack. The chances appear to me to favour this interpretation, because the quartan paroxysm *would* occur on this day, whereas the blackwater might have occurred at any other time. A study of temperature charts (unfortunately few are available), previous to the onset of blackwater, would probably throw light on this point.

I hope, in due time, to be able to collect a number of suitable cases. I have to acknowledge with pleasure the kindness of the Colonial Office in putting the records of this case at my disposal.





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# NUCLEAR VARIATIONS OF THE NEUTROPHILE LEUCOCYTES (ARNETH COUNTS) IN MALARIA AND YELLOW FEVER

BY

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WEST AFRICAN MEDICAL STAFF, GOLD COAST

*(Received for publication 22 July, 1915)*

Considerable interest has recently been evinced in the changes observed to take place in various diseases in the nucleus of the polymorphonuclear leucocyte; and, following Arneth's method of classification of these cells, it has been shown that there is an increase of the cells of Classes I and II in many microbic diseases. The researches of Chamberlain in 1910 first drew attention to the fact that a similar phenomenon is exhibited by persons living in the tropics; and it has recently been suggested by Breinl and Priestley (1914), as the result of their examinations of school children in Queensland, that this change may be 'due to the effects of a tropical climate upon the white race living in the tropics.'

An increase of the percentage of polymorphonuclear leucocytes belonging to Classes I and II has been observed in a great many different diseases. It occurs, according to Cooke (1915), in typhoid, scarlet fever, diphtheria, measles, chicken-pox, erysipelas, tonsillitis, pneumonia, whooping-cough, puerperal fever, tuberculosis, nasal catarrh, and septic conditions, and probably in other diseases also. It is important, therefore, in considering the significance of a shift to the left of the Arneth count, such as that found in Europeans in the tropics, to determine in the first instance the effect on the polymorphonuclear leucocytes of the diseases prevalent in these climates, and especially that of such an infection

as malaria which may lie latent in the body for long periods without exciting active symptoms. In the following pages are embodied the results of the examinations of a number of cases of malarial fever and yellow fever with special reference to the changes occurring in the polymorphonuclear leucocytes.

The blood-films were all stained by Leishman's method. Two hundred successive neutrophile leucocytes were examined in each film, each hundred being counted separately and the two sets of figures being accepted only if they agreed approximately. Nuclear fragments connected by a fine thread were counted as separate; but those joined by a definite band were counted as one. In all doubtful cases the cells were referred to the higher of the two classes to which they might have been assigned.

In making Arneth counts there must always be a very considerable personal variation. Many cells are exceedingly difficult to assign to any particular class, and in such cases different investigators might easily differ in their ultimate decisions. The various estimates of the normal Arneth index, which range from twenty-five to fifty-six, are in themselves a proof of this fact. Allowance must always be made, therefore, for some degree of individual difference in comparing counts made by different observers, and too great importance should not be attached to small degrees of shift. A series of counts by the same observer in different conditions should, however, be strictly comparable. In making the counts referred to below, great pains were taken to try and follow the course of the nucleus before attempting to decide to which class the cell should be assigned. It was assumed that the nucleus was a continuous body with two free ends, and that it should be possible to trace the connexions between the different lobes. This assumption was not always borne out in fact, as leucocytes are occasionally met with even in normal blood in which the nuclear fragments are actually separate from one another. Such cells are probably dead cells; but as they are invariably easy to classify, they do not affect the counts. In my experience the greatest difficulties occurred in films which showed a normal, or nearly normal, count. In those in which there was a marked shift to the left, and these were the most interesting of the series, there was seldom any doubt whatever as to the classes in which the various leucocytes should be placed.



## I. THE RESULTS OF ARNETH COUNTS IN HEALTHY EUROPEANS AND NATIVES

### A. EUROPEANS.

Twenty-nine apparently healthy Europeans were examined at various stages of their tours of service in West Africa (see Table I). The average Arneth index was 51·6; that is, there was a slight shift to the left in the count of about the same degree as that found by Chamberlain and Vedder (average index 46·2) in seventy-two healthy American soldiers in the Philippine service.

Eleven of the subjects had been in residence in the Gold Coast for less than a year, and eighteen for twelve months or longer. The Arneth index in the former averaged 50·6, and in the latter 51·6; a scarcely perceptible increase in favour of those who had been in West Africa for the longer period.

Two of the Europeans had landed at Accra only one and four days respectively before they were examined. In the latter the Arneth count was practically normal (index 41·5), but in the former there was a slight but distinct shift to the left of the count for which there was no apparent cause. He had previously served in West Africa, and had, of course, been in the tropics during the last few days of the voyage before he landed, and had possibly, although not probably, been infected by mosquitos. But in this connexion it should be noted that it is not a very uncommon occurrence for men returning to West Africa to show symptoms of malaria soon after landing; and that this is probably due to their having failed, during their leave of absence, to eradicate from their systems the malarial infections contracted during their previous tour of service.

In a subsequent part of this paper it is shown that in malaria there is a pronounced shift to the left of the Arneth count, and that this phenomenon is observable before the parasites are sufficiently numerous to be found in the peripheral blood, and that it may persist for a considerable time after the patient has apparently recovered. Practically all the apparently healthy Europeans examined had suffered from malarial fever at some time during their tour, and it may be assumed that they had all been repeatedly inoculated with malaria parasites by infected mosquitos. I believe that this is a sufficient explanation of the slight shift to the left of

TABLE I.—Arneth counts in apparently healthy Europeans at Accra, West Africa.

No.	Sex	Length of tour	Arneth classification per cent.					Arneth Index
			I	II	III	IV	V	
1	M.	4 days ... ..	5.5	36.0	41.0	15.5	2.0	41.5
2	M.	1 day ... ..	8.5	45.0	39.0	7.0	0.5	53.5
3	F.	3 months... ..	6.0	30.0	47.5	14.0	2.5	36.0
4	M.	6 „ ... ..	10.0	29.0	37.0	21.0	3.0	39.0
5	M.	6 „ ... ..	5.0	41.0	39.5	13.0	1.5	46.0
6	F.	7 „ ... ..	12.5	38.5	34.5	12.5	2.0	51.0
7	M.	10 „ ... ..	9.5	41.5	37.5	11.0	0.5	51.0
8	M.	10 „ ... ..	13.5	47.0	30.0	9.0	0.5	60.5
9	F.	10 „ ... ..	16.0	47.0	29.5	7.5	—	63.0
10	M.	11 „ ... ..	17.0	38.5	36.5	8.0	—	55.5
11	M.	11 „ ... ..	13.5	46.5	36.0	4.0	—	60.0
12	M.	12 „ ... ..	15.0	38.8	35.0	10.0	1.2	53.8
13	M.	12 „ ... ..	22.0	46.0	29.0	3.0	—	68.0
14	M.	12 „ ... ..	13.0	45.0	37.5	4.5	—	58.0
15	M.	12 „ ... ..	17.0	40.0	35.0	8.0	—	57.0
16	M.	12 „ ... ..	7.5	41.5	36.0	14.0	1.0	49.0
17	F.	12 „ ... ..	4.0	41.0	42.0	13.0	—	45.0
18	M.	12 „ ... ..	10.0	42.5	37.0	9.5	1.0	52.5
19	F.	12 „ ... ..	16.0	39.5	33.5	11.0	—	55.5
20	M.	13 „ ... ..	18.0	37.0	33.5	10.5	1.0	55.0
21	M.	13 „ ... ..	7.5	44.0	40.0	8.0	0.5	51.5
22	M.	13 „ ... ..	18.0	57.0	23.5	1.5	—	75.0
23	F.	13 „ ... ..	9.5	43.5	37.5	9.5	—	53.0
24	M.	13 „ ... ..	13.5	41.0	36.0	9.0	0.5	54.5
25	M.	14 „ ... ..	7.0	33.0	41.0	17.0	2.0	40.0
26	M.	14 „ ... ..	8.0	38.5	42.5	10.0	1.0	46.5
27	M.	15 „ ... ..	5.5	35.0	43.0	14.5	2.0	40.5
28	M.	15 „ ... ..	8.0	24.5	40.0	23.5	4.0	32.5
29	M.	16 „ ... ..	15.0	37.0	35.0	11.0	2.0	52.0
Averages	...	...	11.4	40.2	36.7	10.7	1.0	51.6

the Arneth count found in these subjects. I do not consider that there is at present sufficient evidence in support of the view that this phenomenon is due to the influence of a tropical climate on the white race. The fact that the shift to the left was almost the same in those that had been in West Africa over a year, and in those who had but recently arrived, is, I think, contrary to any such supposition.

It is clear, at any rate, from these examinations that certain individuals may show a normal Arneth count after more than a year's residence in West Africa (see Table I, cases Nos. 25 and 26). Some of the individuals in whom the count approximated most nearly to the normal were men who had spent many years on the 'Coast,' and who might be supposed to have become acclimatised, or to have mastered the conditions of life most suitable for West Africa. Others were individuals who were on the whole better cared for than the majority of their fellows, who had better quarters, and who were to a great extent relieved from the petty domestic worries that are responsible for so much irritation, and probably so many of the ailments, in West Africa.

#### B. NATIVES.

Arneth counts were made in the cases of twenty apparently healthy natives at Accra. All the blood films were taken on the same day, and within the same hour, from twenty labourers who were actually at work at the time when they were called up for examination. The average index worked out at 55·92, and the averages of the percentages of the polymorphonuclear leucocytes belonging to the five classes of Arneth's classification showed a slight shift to the left (see Table II).

There were, however, great variations in the different counts; and the index ranged from 26·5 to 98·5. It is doubtful, therefore, if an average based on such a small number of counts is of any value. A better idea of the actual conditions found is obtained, I believe, by distributing the indices into groups, and plotting a curve to show the percentages of the cases that fall into each group. This has been done in Chart I. It will be observed that the crest of the curve in natives shows a slighter degree of shift to the left from the normal point than that in Europeans; although in the former the average index worked out at 55·92, and in the latter at 51·6.

TABLE II.—Arneth counts in apparently healthy natives at Accra, West Africa.

No.	Arneth classification per cent.					Arneth index
	I	II	III	IV	V	
1	7.0	44.5	38.0	9.5	1.0	51.5
2	10.0	42.0	41.0	7.5	0.5	52.0
3	24.0	52.0	22.0	2.0	—	76.0
4	22.5	51.0	24.0	2.5	—	73.5
5	9.5	37.5	39.5	12.5	1.0	47.0
6	15.0	36.0	39.5	9.5	—	51.0
7	2.5	24.0	48.0	21.5	4.0	26.5
8	10.0	37.0	40.0	13.0	—	47.0
9	13.0	37.0	37.5	12.5	—	50.0
10	80.5	18.0	1.0	—	0.5*	98.5
11	12.5	36.5	38.0	11.0	2.0	49.0
12	10.0	36.5	38.5	13.0	2.0	46.5
13	8.0	34.0	40.0	17.0	1.0	42.0
14	16.0	41.5	33.5	8.0	1.0	57.5
15	14.5	49.0	30.5	6.0	—	63.5
16	18.5	48.0	29.0	4.5	—	66.5
17	14.5	35.5	38.5	11.0	0.5	50.0
18	10.5	35.0	43.5	10.5	0.5	45.5
19	13.5	36.5	39.5	9.5	1.0	50.0
20	31.5	43.5	23.0	2.0	—	75.0
	17.17	38.75	34.22	9.15	0.75	55.92

\* This leucocyte was probably a dead one undergoing chromolysis. I am unable to account for the very high index in this case. The man when seen again a day or two later appeared to be in good health, and no information could be elicited excepting that he had had 'fever a short time ago.' His Arneth index four days later was 64.5.

The shift to the left of the Arneth count in natives at Accra was only a slight one. It was much less pronounced than that discovered by Chamberlain and Vedder in Filipinos (average index 65.8).

There are several factors in the normal life of healthy natives that may have a bearing on the condition of the blood, especially the prevalence of malarial and intestinal infections.

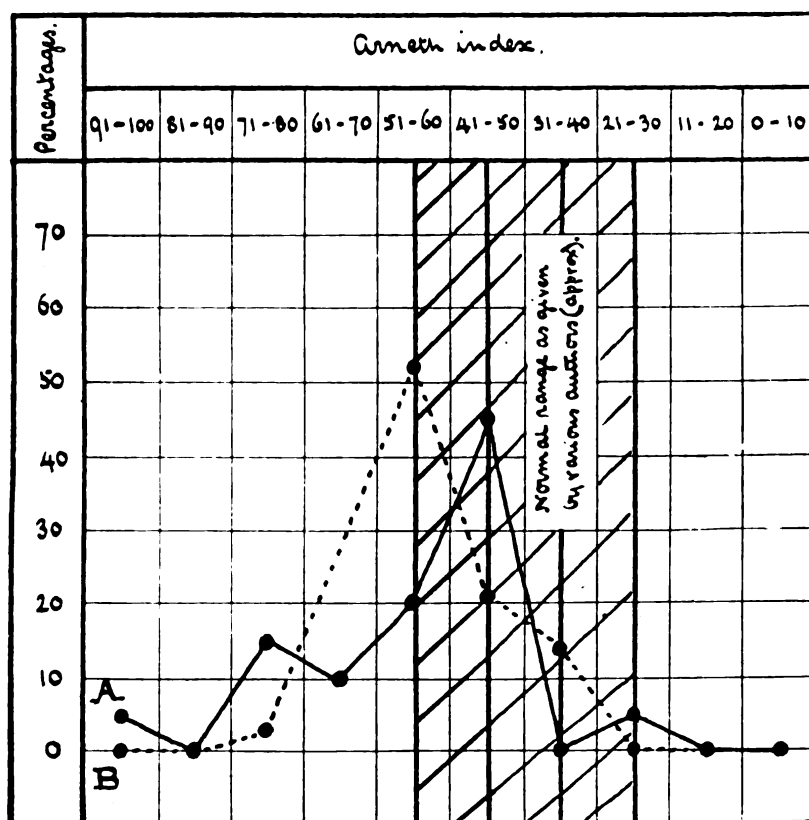


CHART I.—The distribution by groups of the Arneth index in (A) twenty healthy natives, and (B) twenty-nine healthy Europeans at Accra.

It may be assumed that nearly every native harbours intestinal parasites, since ova of various species of worms, *Trichomonas*, and spirochaetes are very generally found in the faeces, and adult worms are recovered at practically every post-mortem examination. Of the blood changes produced by these infections anaemia and eosinophilia are the best known; but Knapp (1915) has recorded that he

has found a shift to the right of the Arneth count in amoebic dysentery and ankylostomiasis.

With regard to malaria, plasmodia are to be found in the blood of all, or almost all, native children; and every native in the course of his normal daily life must be repeatedly inoculated by infected mosquitos. I believe that in natives, as in Europeans, the shift to the left of the Arneth count found in apparently healthy individuals may in most cases be accounted for by such malarial infections. Other diseases may, of course, be responsible in some cases; such, for instance, as tuberculosis, or the mild attacks of yellow fever that scarcely incapacitate the natives at all. But as malarial infections dominate the field in tropical medicine to so great an extent, and as in this disease there is so pronounced a shift to the left of the Arneth count, it is not unreasonable to suppose that in the majority of cases the shift in apparently healthy individuals is due to this cause.

If infections with intestinal parasites (amoebae, worms, *Trichomonas*, spirochaetes, &c.) are proved to cause a shift to the right of the Arneth count, there must be a condition of balance in the cases of most natives in which the shift to the left resulting from malarial infections on the one scale weighs against the shift to the right due to intestinal parasites on the other. And the same condition may occur in latent infections with syphilis and leprosy, diseases in which Knapp also noted a shift to the right.

## II. THE RESULTS OF ARNETH COUNTS IN MALARIAL FEVER

In a previous note (1915) it was pointed out that in malarial fever there is a well marked shift to the left of the Arneth count, and that this phenomenon persists after all parasites have vanished from the blood, and after the patients have recovered from the attacks. At the time this preliminary note was published I had not seen any other recorded observations on the nuclear variations of the polymorphonuclear leucocytes in malarial fever. Recently, however, I have received a copy of the Indian Medical Gazette (March, 1915) containing a paper by Knapp on 'The Significance of Arneth's Leucocyte Count,' in which the author records that he has found a

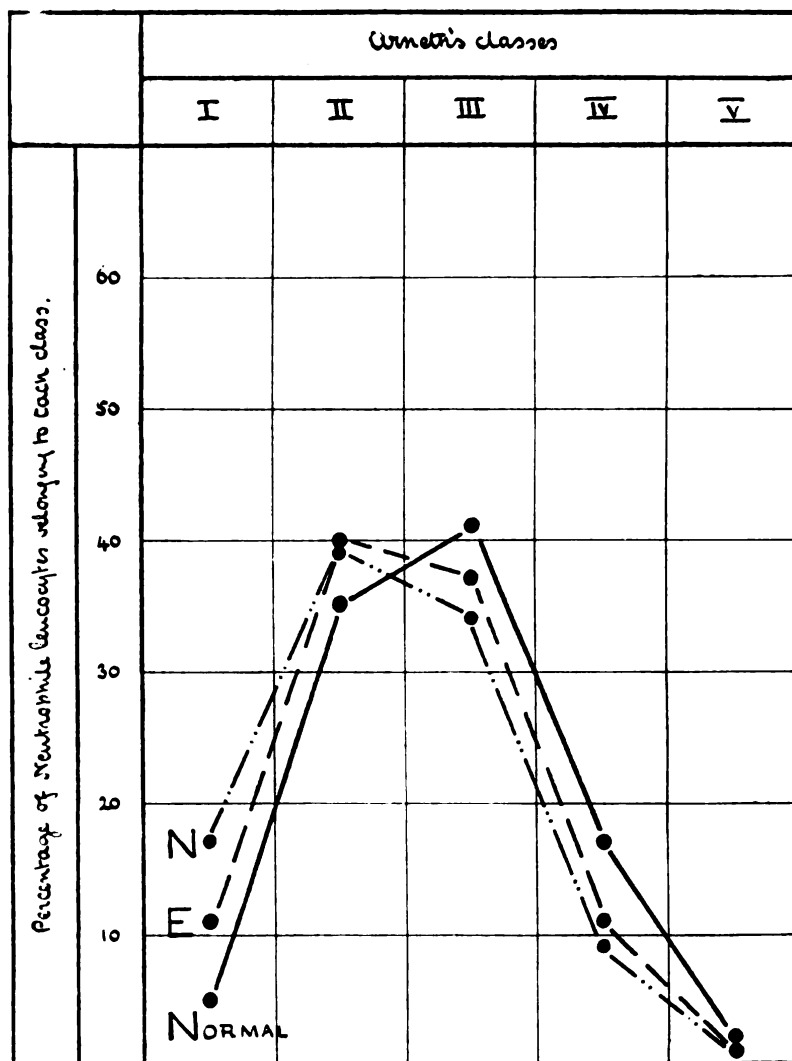


CHART II.—The average Arneth count in (N) twenty healthy natives, and (E) twenty-nine healthy Europeans, at Accra, compared with the normal count in Europe (Arneth)

decided shift to the left in malaria. Knapp states that 'Out of 21 cases, it was marked in 4, definite in 13, and absent in only 4,' that it was, on an average, 'equal in tertian and subtertian fevers, and a single case of quartan gave a similar finding'; and he concludes that 'on the whole it may be fairly said that absence of the left-shift is presumptive evidence against malaria.' Particulars of the counts are given in only two cases: the one, a case of 'Double Tertian,' had an index of (?) 66.9, and the other, 'Subtertian,' an index of 59.4.

During the last few months I have performed Arneth counts in twenty-three cases of malaria in which the diagnosis was confirmed by the finding of the parasites, nine cases of fever in which there was presumptive evidence of malaria, namely an increase of the large mononuclear and transitional leucocytes to 15 per cent. or over, and twelve cases of fever suspected of being malarial but in which neither positive nor presumptive evidence of infection could be found. That is, counts have been made in forty-four cases of proved or suspected malarial fever.

Of the twenty-three cases in which malaria parasites were found, ten were Europeans, one a Syrian, one a half-caste child, and eleven natives. In each case a very marked shift to the left of the Arneth count was found (see Table III). My results were, therefore, much more uniform than those of Knapp, and the shift observed was much more pronounced. In the Europeans the index averaged 84.25, and in two cases was as high as 95.5. In the natives it was even higher; the average being 89.7, and the highest 97.5. *P. falciparum* was the only species of parasite present in nineteen of the cases, and *P. malariae* in two. One case showed both *P. falciparum* and *P. malariae*, and one *P. falciparum* and *P. vivax* (see Table III).

A rough estimation of the number of parasites present in the peripheral blood was made in each case, and the results are shown in the Table as a ratio of the number of red corpuscles to each parasite. An examination of the figures shows that there is no direct relationship between the number of parasites present in the peripheral blood and the degree of deflexion to the left of the Arneth count. The deflexion was greatest in those cases in which the constitutional disturbances were greatest; and, in the case of natives, in children.



TABLE III.—Arneth counts in twenty-three cases of malarial fever.

No.	Race	Nature of the infection	Arneth classification per cent.					Arneth index
			I	II	III	IV	V	
1	European ...	<i>P. falciparum</i> , 1 : 1·4 R.B.C. A few <i>P. malariae</i> . Fatal case	78·0	17·5	4·0	0·5	—	95·5
2	" ...	<i>P. falciparum</i> , 1 : 700 R.B.C. ...	51·0	32·0	16·0	1·0	—	83·0
3	" ...	<i>P. falciparum</i> , 1 : 625 R.B.C. ...	22·0	53·0	23·0	2·0	—	75·0
4	" ...	<i>P. falciparum</i> , 1 : 250 R.B.C. ...	27·0	47·0	24·0	2·0	—	74·0
5	" ...	<i>P. falciparum</i> , 1 : 700 R.B.C. ...	27·5	50·5	19·5	2·5	—	78·0
6	" ...	<i>P. falciparum</i> , very rare. Blackwater fever. Fatal	60·0	27·0	12·0	1·0	—	87·0
7	" ...	<i>P. falciparum</i> , 1 : 714 R.B.C. ...	56·5	39·0	4·0	0·5	—	95·5
8	" ...	<i>P. falciparum</i> , 1 : 830 R.B.C. ...	43·5	46·5	10·0	—	—	90·0
9	" ...	<i>P. falciparum</i> , few. Blackwater fever, first day. Mild case	29·5	45·0	21·5	4·0	—	74·5
10	" ...	<i>P. falciparum</i> , 1 : 8500 R.B.C. ...	41·0	49·0	9·0	1·0	—	90·0
11	Syrian ...	<i>P. falciparum</i> , 1 : 1250 R.B.C. ...	24·0	56·0	17·0	3·0	—	80·0
12	Half-caste ...	<i>P. falciparum</i> , 1 : 20 R.B.C. ...	30·0	53·5	16·0	0·5	—	83·5
13	Native ...	<i>P. falciparum</i> , 1 : 400 R.B.C. ...	67·0	25·0	8·0	—	—	92·0
14	" ...	<i>P. falciparum</i> , 1 : 713 R.B.C. ...	46·0	43·0	11·0	—	—	89·0
15	" ...	<i>P. malariae</i> , very rare ...	24·0	52·5	21·0	2·5	—	76·5
16	" ...	<i>P. malariae</i> , scanty ...	57·0	34·0	9·0	—	—	91·0
17	" ...	<i>P. falciparum</i> (crescents only), 1 : 5000; and <i>P. vivax</i> , 1 : 4100 R.B.C.	32·0	55·0	13·0	—	—	87·0
18	" ...	<i>P. falciparum</i> , 1 : 100 R.B.C. ...	35·0	48·0	16·0	1·0	—	83·0
19	" ...	<i>P. falciparum</i> , 1 : 19 R.B.C. ...	54·5	35·0	10·0	0·5	—	89·5
20	" ...	<i>P. falciparum</i> , very few ...	56·0	41·5	2·5	—	—	97·5
21	" ...	<i>P. falciparum</i> , scanty ...	39·5	53·0	7·5	—	—	92·5
22	" ...	<i>P. falciparum</i> , very rare ...	64·0	33·0	3·0	—	—	97·0
23	" ...	<i>P. falciparum</i> , 1 : 91 R.B.C. ...	54·0	37·5	7·5	1·0	—	91·5
Averages of the counts in the twenty-three cases ...			44·3	42·3	12·4	1·0	—	86·6

The shift to the left was also well marked, but less in degree, in those cases of fever in which there was only presumptive evidence of malarial infections (see Table IV). The index averaged 79·3 in the

TABLE IV.—Arneth counts in cases in which no malaria parasites could be found, but in which the large mononuclear and transitional leucocytes numbered 15 per cent. or over.

Race	Arneth classification per cent.					Arneth index	Differential counts per cent.				
	I	II	III	IV	V		Polymorpho-nuclear	Lymphocytes	Large mono-nuclears and transitionals	Eosinophiles	Mast cells
European ...	59·0	36·5	4·5	—	—	95·5	74·0	11·0	15·0	—	—
„ ...	39·5	47·0	12·0	1·5	—	86·5	61·5	20·5	16·5	1·0	0·5
„ ...	31·5	50·0	17·0	1·5	—	81·5	51·0	31·5	16·5	1·0	—
„ ...	34·0	50·0	15·0	1·0	—	84·0	42·5	37·5	18·0	1·5	0·5
„ ...	8·0	50·5	31·0	10·0	0·5	58·5	56·0	22·5	18·5	2·0	1·0
„ ...	24·0	36·5	28·5	9·5	1·5	60·5	54·0	14·0	20·0	12·0	—
„ ...	33·0	49·0	17·0	1·0	—	82·0	66·0	12·0	21·5	0·5	—
„ ...	33·5	46·0	17·5	3·0	—	79·5	59·5	6·0	34·5	—	—
Native ...	40·0	46·0	13·0	1·0	—	86·0	59·0	25·0	15·0	0·5	0·5
Averages ...	33·6	45·7	17·3	3·2	0·2	79·3	58·2	20·0	19·5	2·0	0·3

nine cases, and ranged from 58·5 to 95·5. No direct connexion between the percentage of the large mononuclear leucocytes and the degree of the shift could be traced. This was not to be expected, as the blood films were taken at different stages of the fever, and it is well known that the percentage of large mononuclear cells is subject to great fluctuations in the course of the disease.

Of the other twelve cases of fever, seven were in Europeans, and five in natives. The seven cases in Europeans were all diagnosed as malarial from their clinical appearances, but no proof of infection could be obtained by the examination of the blood. In West Africa, where practically every European takes five grains of quinine daily, it is often extremely difficult to find evidences of malarial infections in the blood in cases of fever which look like malaria, and which

respond favourably to treatment with somewhat larger doses of quinine. There can be little doubt that the majority of such cases are actually malarial; but they are a source of difficulty and anxiety to the physician, and any sign that would assist in the diagnosis would be of great value. It is interesting, therefore, to note that in all these seven cases there was a decided shift to the left of the Arneth count. The index averaged 71·35; the lowest being 61·5, and the highest 82·0 (see Table V).

TABLE V.—Arneth counts in cases of fever suspected of being malaria, but in which neither positive nor presumptive evidence could be found.

No.	Race				Arneth classification per cent.					Arneth index
					I	II	III	IV	V	
A 1	European	...	...	...	17·5	39·0	32·5	10·0	1·0	56·5
2	"	...	...	...	39·0	41·0	18·0	2·0	—	80·0
3	"	...	...	...	18·0	48·5	29·0	4·5	—	66·0
4	"	...	...	...	33·0	49·0	16·0	2·0	—	82·0
5	"	...	...	...	15·0	46·5	29·5	9·0	—	61·5
6	"	...	...	...	23·0	51·5	23·5	2·0	—	74·5
7	"	...	...	...	30·0	49·0	18·0	3·0	—	79·0
Averages	...	...	...	...	25·07	46·36	23·79	4·64	0·14	71·4
B 1	Native	...	...	...	37·5	49·5	12·0	1·0	—	87·0
2	"	...	...	...	4·0	28·0	50·0	15·0	3·0	32·0
3	"	...	...	...	16·0	53·0	26·0	5·0	—	69·0
4	"	...	...	...	16·5	54·0	27·5	2·0	—	70·5
5	"	...	...	...	7·0	44·0	41·0	8·0	—	51·0
Averages	...	...	...	...	16·2	45·7	31·3	6·2	0·6	61·9

In malarial fever, therefore, there is a marked shift to the left of the Arneth count, which may be extreme in degree in cases accompanied by severe constitutional symptoms, but is still quite definite

even in cases in which there is no proof of the nature of the infection other than the clinical and therapeutic one. In severe cases the majority of the polymorphonuclear leucocytes are of the types included in Class I, and forms with simple horseshoe-shaped nuclei are peculiarly abundant. On examining a blood film from such a case one is at once struck by the uniform and unfamiliar appearance of the polymorphonuclear leucocytes, and the almost complete absence of the familiar normal forms with the nucleus divided into a number of separate lobes joined together by delicate threads.

This effect on the neutrophile leucocytes is observable before the onset of the attack of malaria, and before the parasites are sufficiently numerous to be detected in an examination of the peripheral blood. An example may perhaps be given. On April 21st I examined the blood of a European who had landed at Accra three weeks previously. He had recently returned from leave, and had not taken any quinine for six months, but had enjoyed good health during all this time. I found that there was a marked shift to the left of the Arneth count, and that the index was 61.5; and although no parasites and no pigmented leucocytes could be found, and the percentage of the large mononuclear cells was not higher than that frequently found in healthy Europeans in West Africa, I suggested that it was probable that he had been recently infected with malaria. A few days later he developed a typical attack of malarial fever.

This shift to the left may persist for a considerable time after the attack of malaria is apparently cured. In Table VI the results of five successive Arneth counts on the same individual are shown.

TABLE VI.—Successive Arneth counts in a severe case of malarial fever in a native.

Date	Clinical condition	Arneth classification per cent.					Arneth index
		I	II	III	IV	V	
Jan. 14, 1915...	<i>P. falciparum</i> , 1 to 400 R.B.C....	67.0	25.0	8.0	—	—	92.0
Jan. 15, 1915...	<i>P. falciparum</i> , 1 to 713 R.B.C....	46.0	43.0	11.0	—	—	89.0
Jan. 25, 1915...	Well ... ..	27.5	47.0	22.0	3.5	—	74.5
Feb. 9, 1915...	Well ... ..	20.0	42.5	34.5	3.0	—	62.5
Feb. 24, 1915...	Well ... ..	18.5	43.5	34.0	4.0	—	62.0

This patient, a native, was treated with quinine only during the actual attack of fever; and although he made a rapid and apparently complete recovery, there was still a decided deflexion of the Arneth count six weeks after the onset of his illness.

In West Africa at the present time repeated infection with the parasites of malarial fever must be recognised as one of the normal factors of everyday life alike for the native and the European. The native by his tolerance, and the European by his prophylactic doses of quinine, is able, as a rule, to ward off the actual attack of fever; but the parasites are continually being introduced into the system, and sooner or later they obtain a foothold. Once infected, it is well known how difficult it is to eradicate them completely from the body. Bearing these facts in mind, I think it is only natural that the average native and European in West Africa should show evidences of malarial infection, and I believe that the shift to the left of the Arneth counts found in healthy individuals (see Section I) are to be explained in this manner.

### III. THE RESULTS OF ARNETH COUNTS IN YELLOW FEVER

The early diagnosis of yellow fever is a matter of great difficulty, especially in the mild attacks which occur in natives in West Africa. In the Second Report of the Yellow Fever Commission (West Africa), one of the conclusions arrived at is that 'the extreme practical importance of being able to determine whether a mild case of fever is or is not yellow fever renders it essential that all possible methods should continue to be employed in the clinical study of the disease,' and it is recommended that 'the attention of all workers at this subject should be specially directed to the discovery of a clinical test for yellow fever.'

I was anxious, therefore, to carry out Arneth counts in cases of yellow fever, since this test, should it prove to be of any assistance, would be a very simple one for the physician to employ, as it does not entail any elaborate technique. Unfortunately, the single case of this disease that has occurred at Accra since my arrival was only identified at the autopsy. Recently, however, Dr. J. M. O'Brien has been so kind as to hand over to me a series of blood-films he had collected from cases of yellow fever at Guayaquil, Ecuador; and has permitted me to make use of them for the purposes of this investigation. I wish to take this opportunity of thanking

Dr. O'Brien for his generosity in allowing me to make use of this material.

The slides were from the cases of yellow fever in which Dr. O'Brien (1914) detected a degeneration of the polymorpho-nuclear leucocytes which he believes to be characteristic of the disease. On examining them I found that they had been taken on thirty-five days from seventeen different cases of yellow fever. In seven cases there was only a single blood-film, but in the other ten there were two or more, taken on successive days of the disease. Arneth counts have been made on all the thirty-five films, but I shall limit myself for the present to a consideration of those made on the first days of observation only, the details of the counts on which will be found in Table VII.

TABLE VII.—Arneth counts in yellow fever.

No.	Arneth classification per cent.					Arneth index
	I	II	III	IV	V	
1	43.0	41.0	15.5	0.5	—	84.0
2	48.0	40.5	9.5	2.0	—	88.5
3	67.5	30.0	2.0	0.5	—	97.5
4	69.0	30.0	1.0	—	—	99.0
5	82.0	17.0	1.0	—	—	99.0
6	40.0	40.0	19.0	1.0	—	80.0
7	49.0	44.0	7.0	—	—	93.0
8	58.5	36.5	5.0	—	—	95.0
9	75.0	24.0	1.0	—	—	99.0
10	34.0	50.0	15.0	1.0	—	84.0
11	53.0	43.0	4.0	—	—	96.0
12	20.0	52.5	22.0	5.0	0.5	72.5
13	55.5	34.0	8.5	2.0	—	89.5
14	59.5	36.5	4.0	—	—	96.0
15	49.0	41.0	10.0	—	—	90.0
16	42.5	36.0	20.5	1.0	—	78.5
17	60.0	37.5	2.0	0.5	—	97.5
	53.26	37.26	8.64	0.79	0.02	90.52

The examination showed that there was a profound shift to the left of the Arneth count in yellow fever. The index on the first days represented in the collection of blood-films averaged 90.5, and in some cases actually 99 per cent. of the polymorphonuclear leucocytes belonged to Classes I and II. Even after allowing liberally for a possible personal factor in the counts, such a deflexion is much greater than has been described by other observers in any other disease. As in the severer cases of malaria, a great many of the cells had a simple horseshoe-shaped nucleus, and in one patient these forms constituted 82 per cent. of the polymorphs.

The shift to the left of the Arneth count in yellow fever is more pronounced than it is in malaria. An attempt to show this fact graphically has been made in Charts III and IV. Chart III shows the average Arneth counts in yellow fever, in malaria, and in normal persons in Europe. Chart IV shows the curves produced by plotting the Arneth indices by percentages into ten equal groups in cases of yellow fever, in Europeans suffering from malaria, and in apparently healthy Europeans in Accra.

It has been shown above that in the twenty-three cases of malaria studied at Accra in which the diagnosis was confirmed by the finding of the parasites, the Arneth index averaged 86.6 as compared with 90.5 in these seventeen cases of yellow fever. The difference is one of degree; and as in some of the cases of yellow fever the index was lower than it was in some of the cases of malaria, the index alone could not be used as a means of making a differential diagnosis between these two diseases from an examination of the blood on the first day on which a case happened to be seen.

In those cases of malaria, however, in which the evidence of infection was presumptive only, the average index was found to be 79.3; and in the cases of fever merely suspected of being malarial, it was lower still, namely, 71.35. It is in cases of the latter type that it is important to be able to decide whether or not they are yellow fever. The occurrence of a very high Arneth index in any suspicious case of fever in which no malaria parasites could be found, and in which there was not presumptive evidence of malaria, would, I believe, be strongly in favour of a diagnosis of yellow fever. It will be necessary, before arriving at a definite decision, to

study the condition in mild cases in natives, and to follow the changes in the Arneth counts from the earliest stages of the disease until convalescence is fully established. I hope to be able to do this as soon as I can procure sufficient local material.

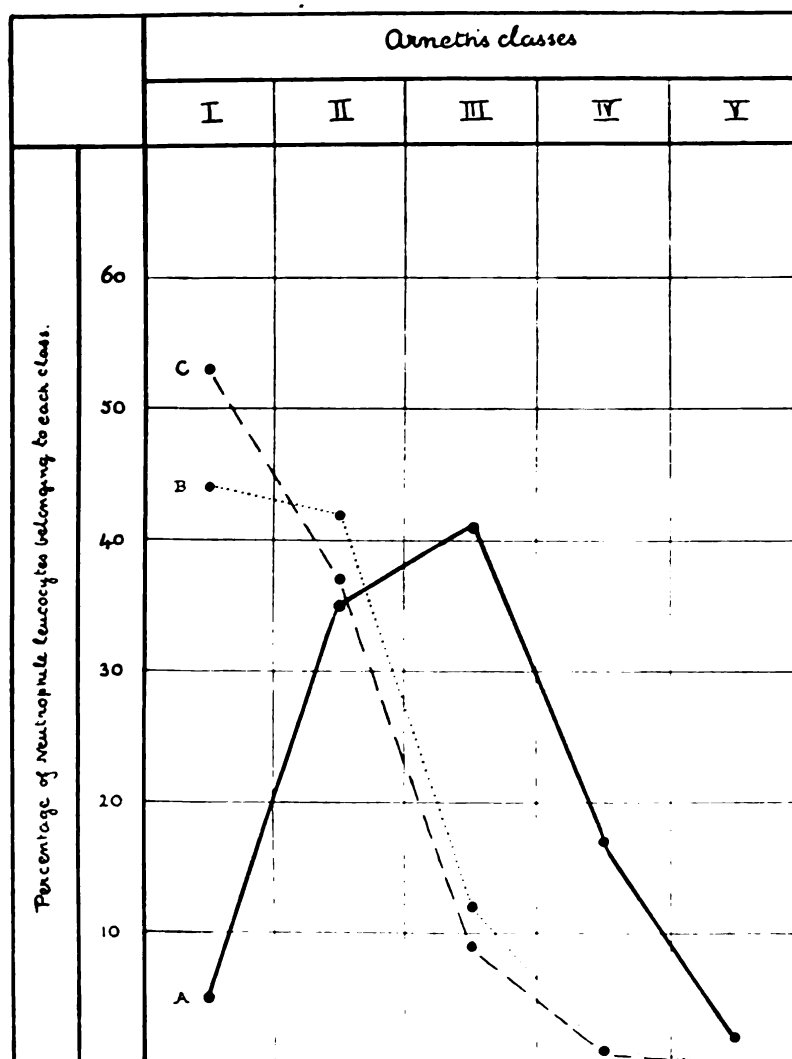


CHART III.—The average Arneth counts in (A) normal persons in Europe (Arneth), (B) twenty-three cases of malarial fever at Accra, and (C) seventeen cases of yellow fever



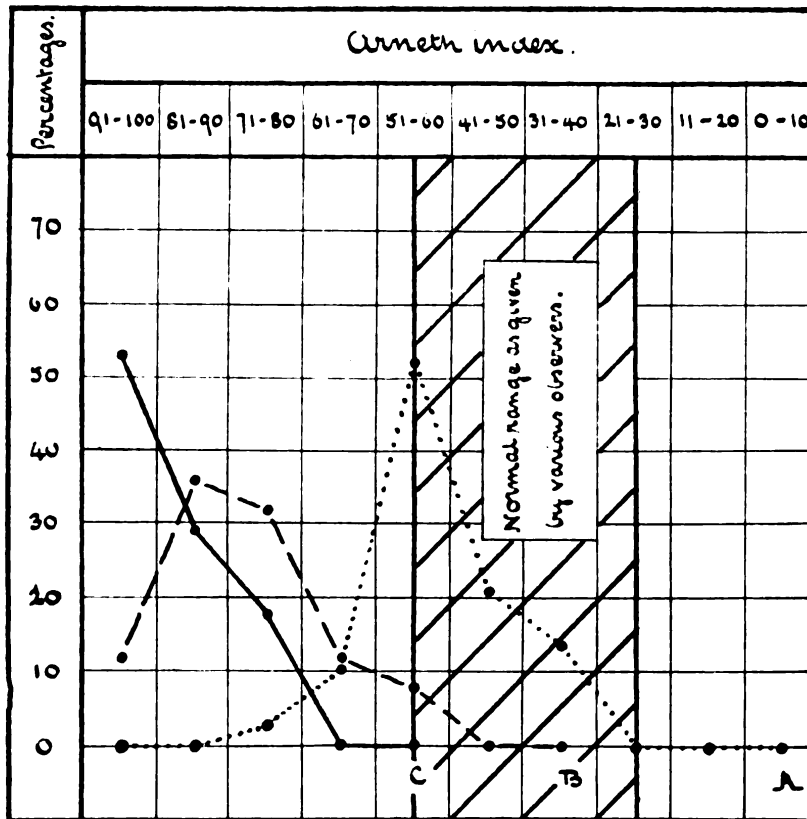


CHART IV.—An attempt to show graphically the degree of the shift to the left in the Arneth counts in (A) healthy Europeans, (B) Europeans suffering from malaria, and (C) yellow fever.

#### IV. THE SIGNIFICANCE OF THE CHANGES OBSERVED IN THE ARNETH COUNTS

A marked shift to the left of the Arneth count has generally been assumed to be an indication of a lowered resistance. As the result of a large number of examinations in various diseases, Arneth concluded that there was a definite relationship between the blood picture and the progress of the disease; and the view adopted by him, and by most subsequent authors, was that the cells of Classes I and II were younger, and less efficient than the cells of the higher classes; and that therefore the degree of the shift to the left was an index of the patient's resistance. Chamberlain (1914) summarises this view by saying 'a high Arneth index (excess of Classes I and II) goes hand in hand with a low resistance, or with a high degree of toxic and bacterial absorption which is bringing about the destruction of the actively phagocytic leucocytes (Classes III and IV).'

There is, however, little evidence in support of the assumption that the neutrophile leucocytes of Classes I and II are less actively phagocytic than those of the higher classes. Many observers have failed to detect any difference in experiments carried out *in vitro*; and I have recorded elsewhere a case of fatal malarial fever in which the leucocytes had ingested a large number of parasites, making it possible to gauge their phagocytic activity *in vivo*, and have shown that in this case the polymorphonuclear leucocytes of Class I were certainly not less efficient than those of the higher classes.

Breinl and Priestley (1914) have, therefore, advanced the view that 'the Arneth picture is an expression of the functional activity of the leucopoetic system, especially the bone-marrow, rather than that of phagocytic activity.' They dissent from the view of Chamberlain and Vedder that the shift to the left observed in Filipinos may be an indication of a lowered resistance to infections on the part of the native races, and attribute the similar phenomenon found in North Queensland children to purely climatic influences. They state that in their experience there is amongst the children in Townsville, who show a decided shift to the left of the Arneth count, 'no greater susceptibility to infectious diseases than amongst the same class in Europe.'

In West Africa the healing powers of the natives are famous, and it is scarcely credible that the slight shift to the left of the Arneth count observed in them can be an indication of a lowered resistance to infection. As I have already stated, I believe that in West Africa the shift in apparently healthy individuals may be accounted for by infections with malaria parasites which do not necessarily culminate in an attack of 'fever.'

The very pronounced shift to the left observed in confirmed cases of malarial fever at Accra may be considered as supporting the view of Breinl and Priestley that the phenomenon is an indication of the functional activity of the leucopoetic system; for on this hypothesis it is in such diseases as malaria, which profoundly affect the spleen and the bone-marrow, that one might expect to find a marked shift to the left indicating a morbid activity of these tissues. The Arneth index was highest in those cases which showed the greatest constitutional disturbances, and not necessarily in those that had the largest number of parasites in the peripheral blood. In yellow fever, another disease accompanied by severe constitutional disturbances and a profound toxæmia, the Arneth counts showed a shift to the left that was often extreme in degree.

The blood-films from yellow fever cases that were examined, were some of those from which O'Brien (1914) described a degeneration of the polymorphonuclear leucocytes which he considered to be characteristic of the disease. Some of the abnormal forms he interpreted, correctly I believe, as being dead cells. Identical forms were described by Dr. Mary Rowley (1907) in a fatal case of aortic and mitral disease with anaemia; and this author was able to bring forward evidence in proof of the fact that these cells had been killed. Similar polymorphonuclear leucocytes are seen occasionally in the blood of normal persons; but they are relatively much more abundant in acute cases of malaria and in yellow fever. It is probable that in these diseases the profound toxæmia causes a great destruction of the circulating polymorphonuclear leucocytes, and thus leads to a relative increase in the peripheral blood of the young cells—namely, those belonging to Arneth's Classes I and II, and a corresponding shift to the left of the count.

The increase in the percentage of young cells in the peripheral blood might result from (1) the elimination of the cells of the higher

classes by the selective action of the toxins as suggested by Chamberlain, or (2) a flooding of the blood with newly-formed elements produced by the activity of the leucopoetic system. As a leucocytosis is frequently observed in blood in which there is a marked shift to the left of the Arneth count, and as there is really no reason to suppose that the cells of the higher classes are the more actively phagocytic, and so the more liable to be destroyed by a high degree of toxic and bacterial absorption, I am inclined to think that the latter explanation is the correct one.

It might be expected, then, that in an intense infection there would be developed an immediate shift to the left of the Arneth count, increasing rapidly in degree as the toxaemia deepened, and tending to diminish as recovery took place.

#### SUMMARY

1. A slight shift to the left of the Arneth count is found in the blood of healthy Europeans in West Africa.
2. There is a marked shift to the left in malarial fever which is evident not only during the attack, but also before its onset, and for a considerable time after convalescence is established.
3. It is probable that the abortive inoculations with malaria parasites by infected mosquitos, which are a part of the daily life in many parts of West Africa, are sufficient to account for this shift in apparently healthy Europeans, without postulating a specific action of the climate on the white races living in the tropics.
4. In yellow fever there is a great shift to the left of the Arneth count.
5. It is suggested that the changes observed in the Arneth counts are due to toxaemia causing a destruction of the circulating polymorphonuclear leucocytes, and a flooding of the blood with young cells liberated by the activity of the leucopoetic system.

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# BABESIASIS AND TRYPANOSOMIASIS AT ACCRA, GOLD COAST, WEST AFRICA

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## BABESIASIS

## A. BABESIASIS OF CATTLE AND SHEEP

In a previous paper (1914) I gave a preliminary account of the occurrence of babesiasis in Nigeria, and described three species of parasites found in the blood of cattle and sheep in that country. Similar observations have been made by Bouet (1908) on the Ivory Coast, and by Broden and Rodhain (1909) in the Congo; the former recording the occurrence of *P. parvum*, *P. mutans* and *P. bigeminum*, and the latter *P. mutans* and *P. bigeminum*. From these observations it is evident that piroplasms are common on the West Coast, and it may therefore be of some interest to record for comparison the results of the examinations of domestic animals at Accra, in the Gold Coast, since hitherto but little attention has been directed to these parasites in British West Africa.

For the purposes of this investigation blood films were obtained

from the Accra slaughter-house. Hump-backed cattle, straight-backed cattle, sheep, pigs, and goats are slaughtered daily at Accra. Many of the animals are bred locally, but others come from a distance, and no doubt bring with them their parasitic infections. The hump-backed cattle, as in Nigeria, are bred in the north, and are driven south in herds during the dry season until they reach the coast towns. On the journey they are exposed to the attacks of ticks and tsetse-flies, and debilitated by fatigue, they are probably but feebly resistant to parasitic infections. The straight-backed cattle, on the other hand, are mostly bred in the southern parts of the Gold Coast, and the majority of those examined were either Accra or Addah animals; they had not, at any rate, trekked down from the north like the hump-backed cattle. A considerable number of the sheep had also come to Accra from a distance; but the majority of the goats, and probably all the pigs, had been bred in the neighbourhood.

A hundred animals of each kind were examined, namely hump-backed cattle, straight-backed cattle, sheep, pigs, and goats. Only a single blood film was obtained from each animal, so that the percentages of infections found are probably considerably below those actually occurring. In this way piroplasms were found in 53 per cent. of the hump-backed cattle, in 40 per cent. of the straight-backed cattle, and in 21 per cent. of the sheep (Table I). None were found in the pigs and goats.

TABLE I.—The results of the examination of 500 domestic animals for babesiasis.

Host	Number examined	Number infected with Piroplasms	<i>B. bigemina</i>	<i>T. mutans</i>
Cattle, hump-backed breed ... ..	100	53	7	49
Cattle, straight-backed breed ... ..	100	40	3	38
Sheep ... ..	100	21	—	21
Pigs ... ..	100	—	—	—
Goats ... ..	100	—	—	—
Totals ... ..	500	114	10	108

So far as could be ascertained the infections appeared to be benign, but as the animals had frequently concurrent infections with other parasites, especially trypanosomes, it was difficult to determine this point. For instance, forty-seven of the fifty-three hump-backed cattle found to harbour piroplasms were infected with trypanosomes also. In the case of the sheep no trypanosomes were found associated with the piroplasms, and in these animals no clinical symptoms of disease were observed.

Various abnormal conditions of the red corpuscles were found in the blood films, notably a coarse basophilia. Anaplasma-like bodies were present in many cases, but as these are common in healthy animals they can be of no special significance in this connexion. In the blood films from the cattle, especially the straight-backed breeds, there were generally to be found oval or rounded bodies about the size of a red blood corpuscle that stained a pale blue colour. A few granules that stained similarly to chromatin were present in these bodies. These basophile cells were similar to those described by Castellani (1912) as occurring in man in cases of yaws, psoriasis, lichen, acne, severe anaemia, &c.; and they were probably, as he concludes, 'red cells undergoing degenerative changes.' Although piroplasms were present in the blood of most of the cattle in which they were detected, there does not appear to be any direct connexion between them and these parasites, since they were not constantly associated. The basophile cells may, however, have been an indication of the anaemia that is frequently induced by piroplasmosis, although, as has already been stated, the infections appeared otherwise to be benign.

Two species of piroplasm were encountered in the blood films, the one a large pyriform parasite, and the other a small oval or rod-shaped organism. Both types were found in cattle, and the latter in sheep also. The third species found in Nigeria, a large parasite with forms in which the chromatin was divided into six or seven masses, was not found in the Gold Coast.

Owing to the lack of unquestionably uninfected animals for experiments, it has not been possible to attempt to determine the ticks that transmit these parasites. Experiments designed to decide this point could not be conclusive if carried out with locally bred animals in the Gold Coast because of the high percentage of natural

infections in the cattle and sheep, the benign nature of the infections, and the extreme rarity of the parasites in some cases. Some ticks collected from the animals were, however, sent home to England, and were very kindly identified by Prof. Nuttall. The species from both cattle and sheep were the same, namely, *Boophilus*, *Amblyomma variegatum*, and *Hyalomma aegyptium*; and presumably one or more of these transmits the piroplasms.

#### *Babesia bigemina*

The larger species of piroplasm appeared in the blood as oval or pear-shaped bodies of considerable size. Pairs of pyriform bodies were not uncommon, and when found were generally seen to occupy the greater part of the enveloping erythrocyte. There can be little doubt that these parasites should be identified as *Babesia bovis* or *bigemina* (*Piroplasma bigeminum*). In the straight-backed cattle the morphology of the parasites was similar to that found in dwarf cattle in Nigeria (1914); but in the hump-backed cattle the pyriform pairs were somewhat smaller, and their nuclear structure was less distinct. In one hump-backed beast a considerable number of the parasites were notably amoeboid.

This type of *Babesia* was found in 7 per cent. of the hump-backed cattle, and in 3 per cent. of the straight-backed breed. They were never very numerous, and in four of the ten animals in whose blood they were detected they were associated with the smaller type of parasite to be described immediately.

#### *Theileria mutans*

The smaller type of piroplasm was identical with that found in Nigeria. At Accra it was present in 49 per cent. of the hump-backed cattle, in 38 per cent. of the straight-backed cattle, and in 21 per cent. of the sheep. It was, therefore, a very common parasite of the animals brought to the Accra slaughter-house, just as it was of the domestic animals in Nigeria. In several of the hump-backed cattle, and a few of the sheep, the infection was a heavy one; but in the straight-backed cattle the parasites were as a rule scanty or rare. Certainly in the majority of the animals the infection appeared to be benign.

The parasites were very simple but remarkably pleomorphic organisms, appearing as ring-shaped, oval, horseshoe-shaped, and



rod-like bodies, and characteristic cross-forms. Examples of all these different forms were figured in my account of the Nigerian infections, so that it will be unnecessary to illustrate the present description. Since then I have had opportunities of examining smears from the spleen in cases of several infected animals, but up to the present I have not detected the presence of Koch's 'blue bodies' in any of them. Such a parasite, according to the classification of França, would have to be included in the genus *Theileria*, and for this reason, in describing its occurrence in Nigeria, I provisionally identified it as *Theileria parva*, stating at the same time that Koch's 'blue bodies' had not been found, and that the infections appeared to be benign. Minchin (1912), however, considers that 'the diagnosis of the genus *Theileria* given by França would appear to apply to *B. mutans* rather than to *T. parva*,' and states that 'a confusion has arisen between two parasites very similar as regards the appearances they present in the blood, but differing in every other respect, namely, *Theileria parva*, the true parasite of "East Coast fever" of cattle, and *Babesia* (*Piroplasma*) *mutans*, also found in cattle. In both parasites alike the characteristic cross-forms appear in the blood. In *Theileria parva*, however, the cross-forms are an aggregation of four distinct gametocytes which have invaded the same corpuscle, while in *Babesia mutans* the cross-forms are produced by quadruple fission of an ordinary multiplicative individual.' The parasite described from Nigeria, and that found recently in the Gold Coast, showed cross-forms unquestionably produced by fission, and not by the aggregation of four distinct gametocytes in a single cell. It should therefore be identified, according to Minchin, as *B. mutans*. The facts that the infections appear to be benign, and that Koch's 'blue bodies' have not hitherto been detected in smears from the spleen of infected animals, tend also to prove that the parasite is not *T. parva*. Inoculation experiments would assist the diagnosis, as *T. parva* is not inoculable; but for the reasons given above these could not be conclusive if carried out in this country. At the same time, an organism characterised by the occurrence of bacilliform or rod-shaped parasites, and multiplicative forms in the shape of a cross, cannot well be assigned to the genus *Babesia*, and it will probably be best for the sake of clearness to identify this small piroplasm as *Theileria* (*Babesia*) *mutans*.

## B. CANINE BABESIASIS

Up to the present only a single case of canine babesiasis has come under my notice at Accra. The dog found to be infected had been brought into the Colony only six weeks previously. He was feverish, much wasted, and very anaemic. The right eye was dimmed, and there was a profuse watery discharge from the nose. On examining the blood, large numbers of piroplasms were found; many extremely amoeboid, but others showing the pyriform outline and the association in pairs characteristic of *Babesia canis*.

Imported dogs are known to suffer on the Gold Coast from a disease characterised by severe anaemia and rapid emaciation which does not appear to be a trypanosomiasis. Possibly some of the cases of this disease are due to babesiasis. It is the common experience that in Accra dogs become infested with ticks, no matter how carefully they are tended, and it would not therefore be surprising if babesiasis were found to be a prevalent disease.

No case of equine babesiasis has as yet come under my notice in the Gold Coast, although this disease is known to occur in Nigeria and in the neighbouring colonies.

## C. A PIROPLASM, *NUTTALLIA DECUMANI*, n. sp., OF THE BROWN RAT (*MUS DECUMANUS*). Pl. XXXVI, figs. 1-14

In the blood of a few brown rats (*Mus decumanus*) that had either been sent to me for examination by the Medical Officer of Health for Accra, or had been caught in the laboratory itself, unpigmented parasites were found in the erythrocytes. Four out of twenty rats recently examined were found to be infected; that is, a proportion equal to 20 per cent.

From two of the rats only a single blood-film was obtained, and in both of these the parasites were rare. In the other two the infections were heavier, and as I was able to keep the animals in captivity, it was possible to make a more thorough study of the morphology of the parasites. Both these rats were young animals, and both showed in their red corpuscles, in addition to the piroplasms, the bodies known as *Grahamella*. The first was caught on January 10th, 1915, and was kept under observation until February 15th, the thirty-seventh day, when it was accidentally killed. When caught, the examination of the blood showed the presence of

*Grahamella* and Jolly bodies, but no piroplasms. A few days later one or two piroplasms were detected, and by the fifteenth day a considerable number were present, although the infection was never a heavy one, and the parasites had always to be searched for most carefully. From this date the parasites gradually diminished in numbers, so that by the twenty-sixth day they were rare. They were still present on the thirty-seventh day when the rat was killed. The *Grahamella* bodies were not found after the thirteenth day. The second rat was caught on April 27th, and is still under observation. Piroplasms were found in the blood on the first day, and continued to be present up to the tenth day, but have not been detected since. They were always rare, and it was only after prolonged search that they were found. The *Grahamella* bodies have vanished from the blood of this rat also since it has been under observation.

The forms of the parasite most commonly seen consisted of a somewhat irregularly shaped chromatin mass, and an amoeboid or ring-shaped protoplasmic body. The smallest parasite seen was a minute dot of chromatin to which a very small ring of blue-stained material appeared to be attached, but even at this early stage of development there was a paler area or vacuole in the protoplasm (Pl. XXXVI, figs. 1 and 5). More mature parasites were often of considerable size, and great irregularity of outline. The chromatin was in many cases drawn out into a number of processes or separated into two or more distinct masses, and the protoplasm was freely vacuolated or extended in a number of delicate threads. Other parasites were signet-ring-shaped, and closely resembled the characteristic forms of *Plasmodium falciparum*. Sometimes there were two chromatin dots at opposite sides of the ring. None of the parasites was pigmented. The red corpuscles did not appear to be affected by the presence of the parasites, and were neither obviously enlarged nor stippled.

After long search a few specimens were found in which division had taken place, resulting in the production of four lanceolate parasites arranged in the form of a cross. Each of these bodies contained a nucleus which was generally situated near the middle, and in addition a second minute chromatin dot could usually be seen at the distal end. No pairs of pyriform bodies were found.

Subcutaneous inoculations of blood containing these parasites

were made into three white rats, but without transmitting the infection. A single attempt to convey the parasites to another brown rat, by means of ticks taken from the body of an infected animal, was also unsuccessful.

This parasite would seem to belong to the genus *Nuttallia*, and as I am unable to find any reference to a previous description of it, I would suggest the name *Nuttallia decumani* for it, should it prove to be a new species.

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#### TRYPANOSOMIASIS

Three types of trypanosomes were met with at Accra, namely, (1) a polymorphic parasite, (2) a large monomorphic parasite with a long free flagellum, and (3) a short monomorphic parasite without a free flagellum. These three types are the same as those found in Nigeria, and which I have elsewhere identified as (1) *T. pecaui* (*T. brucei* of Uganda, *T. ugandae*), (2) *T. vivax*, and (3) *T. pecorum* (*T. congolense*). In dried blood-films, such as those obtained from the slaughter-house, morphological features had to be relied on for the identification of the trypanosomes. Such data are, of course, insufficient to distinguish the more closely allied species; but as the small number of inoculations into animals that were made seemed to confirm these diagnoses, they are probably correct.

##### A. TRYPANOSOMES FOUND IN BLOOD FILMS OBTAINED FROM THE ACCRA SLAUGHTER-HOUSE

Blood-films were examined from 500 animals slaughtered at Accra, namely, 100 each from hump-backed cattle, straight-backed cattle, sheep, pigs, and goats. The films were the same as those

used in the examinations for Babesiasis, of which an account has already been given; and the remarks made there with regard to the sources from whence the animals came should be taken into consideration with reference to the trypanosome infections. In Accra itself tsetse flies are exceedingly rare. A few are caught every year, but it is generally supposed that these have found their way into the town in railway carriages or waggons. Within a few miles, however, tsetse flies are numerous; and any animals coming from a distance must be exposed to the attacks of these insects for the greater part of their journey. It is probable, therefore, that the majority of the trypanosome infections described had been contracted before reaching Accra.

**HUMP-BACKED CATTLE.** Trypanosomes were found in 92 per cent. of the hump-backed cattle; and as only a single blood-film was examined from each animal, this proportion is more probably below the mark than above it. In the majority the parasites were very numerous. Trypanosomes of the type of *T. vivax* were present in 76 per cent., *T. congolense* in 28 per cent., and *T. pecaui* in 12 per cent. (see Table II). Sixty-nine of the hundred animals examined were infected with a single type of trypanosome, fifteen with *T. vivax* and *T. congolense*, four with *T. vivax* and *T. pecaui*, three with *T. congolense* and *T. pecaui*, and one was the host of all three types, *T. vivax*, *T. congolense*, and *T. pecaui*.

TABLE II.—Trypanosome infections found in animals killed at the Accra slaughter-house.

Host	Number examined	Number infected with trypanosomes	Percentages infected with		
			<i>T. vivax</i>	<i>T. congolense</i>	<i>T. pecaui</i>
Cattle, hump-backed ...	100	92	76	28	12
Cattle, straight-backed ...	100	18	14	6	1
Sheep ... ..	100	4	3	2	—
Pigs ... ..	100	5	—	5	—
Goats ... ..	100	1	1	1	—
Totals ... ..	500	120	18.8	8.4	2.6

Of all the animals slaughtered at Accra the hump-backed cattle have the longest journey to come, and this fact probably accounts for the great number of them found to harbour trypanosomes. Even as far north as Kumasi, I understand, almost all are found to be already infected with these parasites.

**STRAIGHT-BACKED CATTLE.** Trypanosomes were found in 18 per cent. of the straight-backed cattle. The parasites were generally scanty, and were often detected only after a long search. *T. vivax* was found in 14 per cent., *T. congolense* in 6 per cent., and *T. pecaui* in 1 per cent. Fifteen of the animals had a single infection, and three harboured both *T. vivax* and *T. congolense*.

Straight-backed cattle are bred in the southern parts of the Gold Coast, and many of those examined at Accra had not been brought from any great distance. The relatively low percentage of trypanosome infections, and the rarity of the parasites in the blood, should, probably, be correlated with this fact. Some of these animals were, however, of the dwarf breed peculiar to West Africa, and it should be remembered that, as I have suggested elsewhere (1913), they may possess a partial immunity to *T. vivax* at any rate.

**SHEEP.** Four of the hundred sheep examined were found to be infected with trypanosomes, but in each case the parasites were rare. *T. vivax* was found in 3 per cent., and *T. congolense* in 2 per cent. Three sheep had a single infection, and one harboured both species of trypanosome.

**PIGS.** Five out of the hundred pigs examined were found to be infected with trypanosomes, and in each case the type of parasite was *T. congolense*.\* The parasites were generally very rare.

**GOATS.** Only one of the goats was found to harbour trypanosomes, and this animal had a double infection—*T. vivax* and *T. congolense*. It is somewhat remarkable that so few infections should have been found in goats, since these animals have been credited with being a natural reservoir for *T. vivax*, the trypanosome most frequently met with at Accra.

*T. pecaui* (*T. brucei* of Uganda, *T. ugandae*)

Polymorphic trypanosomes were found in thirteen animals (2·6 per cent.), namely, in twelve hump-backed cattle, and in one

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\* Subsequently a pig was examined in whose blood trypanosomes of the *T. pecaui* type were present.

straight-backed cow. This type of trypanosome was the most uncommon of the three forms occurring at Accra. It is the most fatal to domestic animals in Nigeria, and so probably in the Gold Coast also. In the hump-backed cattle it was often associated with other trypanosomes: four times with *T. vivax*, four times with *T. congolense*, and once with both *T. vivax* and *T. congolense*.

Morphologically the trypanosomes resembled *T. pecaui* or *T. brucei* of Uganda, both long and slender, stumpy, and intermediate forms were found in each case, but forms with posterior nuclei were not seen. It is impossible, therefore, to exclude the possibility that some of the trypanosomes might have been *T. gambiense*. The occurrence of posterior nuclear forms is, however, very irregular in cattle infected with trypanosomes which, when subinoculated into rats and guinea-pigs, show high percentages of these forms; and at the periods during which the parasites are scanty, as they were in all the slaughter-house cases, it is generally impossible to find them at all. Too much importance should not, therefore, be attached to the absence of these forms, especially when the materials examined are restricted to dried blood-films.

In its behaviour in the tsetse fly, *T. pecaui* is said to differ from the other polymorphic trypanosomes, development taking place in the gut and proboscis, instead of in the gut and the salivary glands. Confirmation of this observation is desirable; but in any case, in dealing with these slaughter-house films the exogenous cycle of development had to be ignored, and it was impossible to carry identification beyond the point at which the trypanosomes were assigned to what is sometimes known as the '*T. brucei* group.'

In one instance, blood containing this type of trypanosome was obtained from a hump-backed ox, and inoculated subcutaneously into a white rat and a guinea-pig. The rat first showed trypanosomes in its blood on the tenth day, and death took place on the nineteenth day. The parasites were at first very scanty, but gradually increased in numbers up to the day of death; and in the latter stages of the disease, forms with posteriorly placed nuclei were numerous. The guinea-pig did not become infected, but with two others was successfully inoculated with blood taken from the rat. In these three guinea-pigs the incubation periods were respectively twenty, nineteen, and seven days; and the duration of the disease thirty-four, twenty-five, and thirteen days.

Measurements were made of 200 trypanosomes from the blood of the rat (see Table III, and Chart I). The average length was  $27.66 \mu$ ; the longest individual measuring  $39 \mu$ , and the shortest  $15 \mu$ . This trypanosome then corresponded with *T. pecaudi* (*T. brucei* of Uganda), both in its morphology and in its pathogenicity to rats and guinea-pigs.

TABLE III.—Measurements in length of *T. pecaudi* (cattle strain).

Animals	Day of the disease	Number measured	Length in microns		
			Average	Minimum	Maximum
Rat ... ..	1	20	27.65	16	33
Rat ... ..	2	25	27.72	17	37
Rat ... ..	3	20	26.25	16	35
Rat ... ..	4	25	32.64	26	39
Rat ... ..	5	20	26.65	18	33
Rat ... ..	6	25	26.88	16	35
Rat ... ..	7	20	28.2	16	38
Rat ... ..	8	25	27.2	15	39
Rat ... ..	9	20	24.8	16	35
		200	27.66	15	39



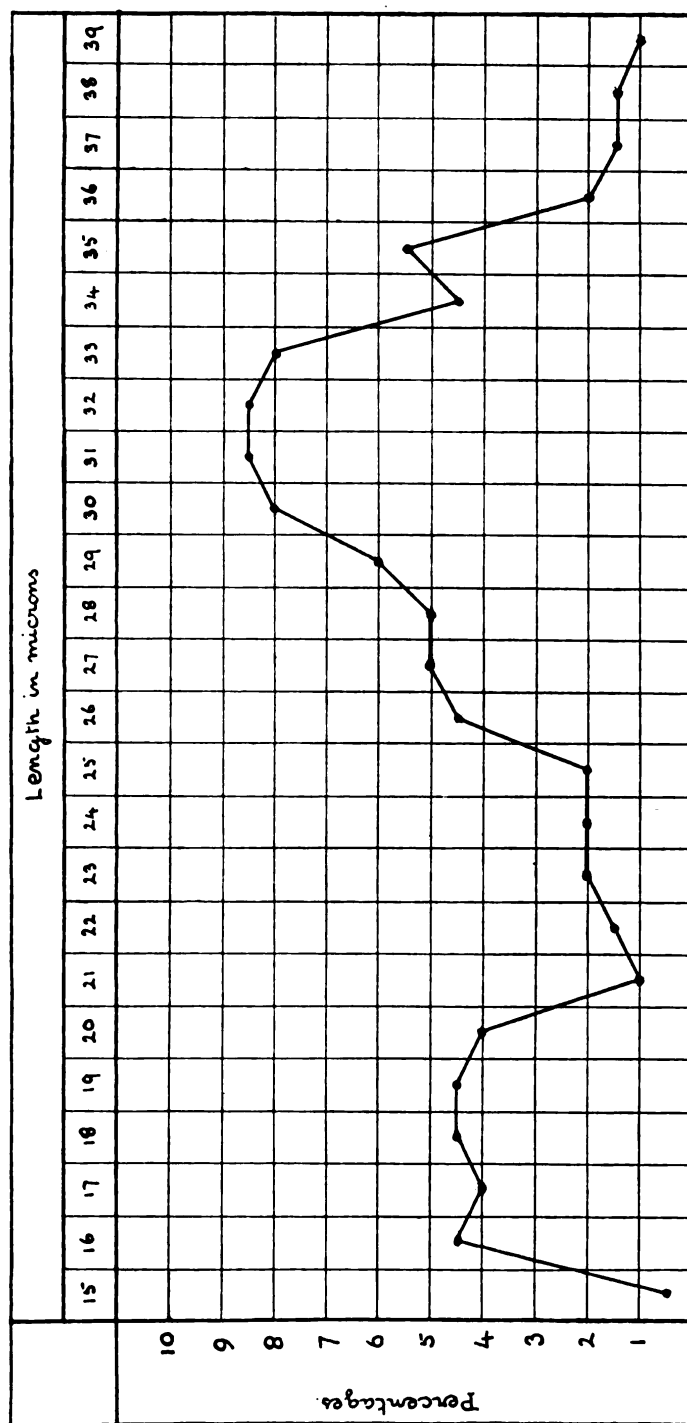


CHART I.—The distribution, by percentages, in length of *T. percaudi* (cattle strain).

*T. vivax*

Trypanosomes morphologically identical with *T. vivax* were present in ninety-four (18·8 per cent.) of the animals examined, namely, in seventy-six hump-backed cattle, fourteen straight-backed cattle, three sheep, and one goat. They caused by far the greatest number of the trypanosome infections met with at Accra, and in many of the animals, especially the hump-backed cattle, were present in the blood in enormous numbers.

Blood from hump-backed cattle heavily infected with these trypanosomes was inoculated into two white rats, two guinea-pigs, and one rabbit, but without result. The insusceptibility of the smaller laboratory animals, taken in conjunction with the very characteristic morphology of the parasites, is sufficient evidence for the identification. Measurements were, however, made of one hundred trypanosomes, fifty from hump-backed cattle, and the same number from straight-backed cattle (see Table IV). The average length was found to be 22·64  $\mu$ , and the range from 18  $\mu$  to 29  $\mu$ . These measurements, and the curve shown in Chart II, are consistent with the identification *T. vivax*.

TABLE IV.—Measurements in length of *T. vivax* from cattle.

Host	Number measured	Distribution according to length in microns												Average
		18	19	20	21	22	23	24	25	26	27	28	29	
Cattle, hump-backed, No. 451 ...	25	—	1	2	4	4	8	5	1	—	—	—	—	22·40
Cattle, hump-backed, No. 630 ...	25	—	1	—	1	4	9	6	1	2	1	—	—	23·32
Cattle, straight-backed, No. 589	25	1	—	2	2	5	5	3	4	1	1	—	1	23·16
Cattle, straight-backed, No. 734	25	—	—	1	5	5	7	4	3	—	—	—	—	22·68
	100	1	2	5	12	18	29	18	9	3	2	—	1	22·64

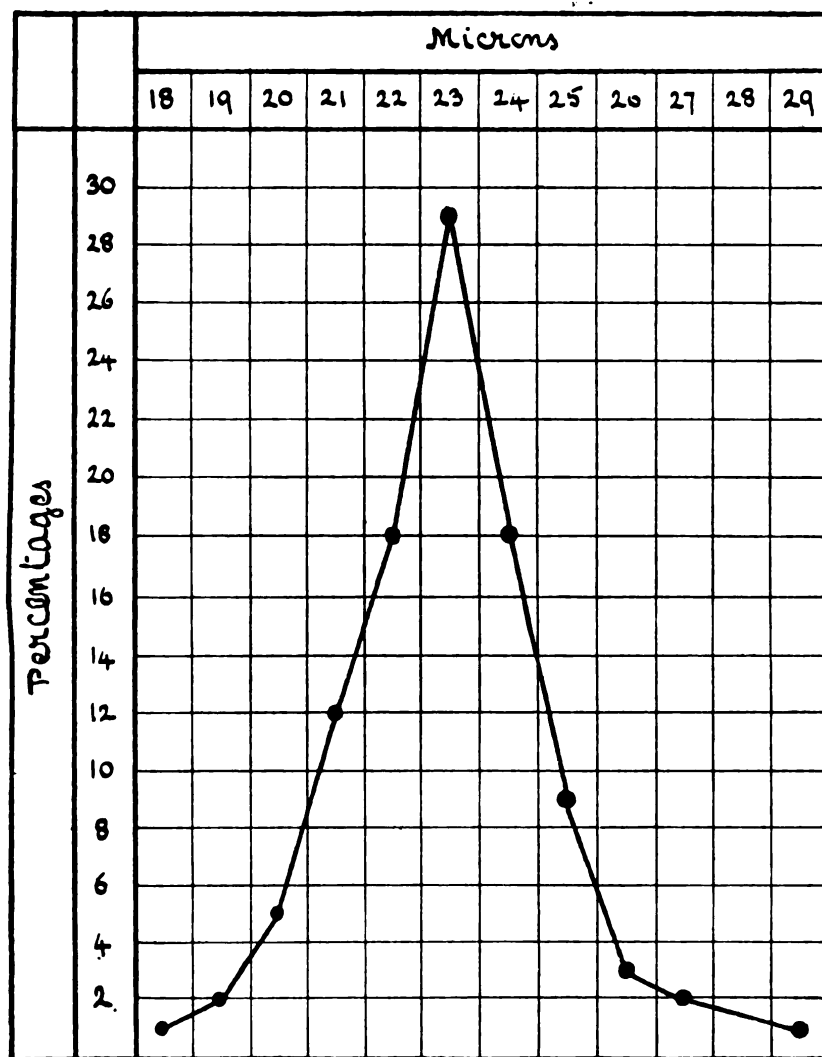


CHART II.—The distribution, by percentages, in length of *T. vivax* from cattle.

*T. congolense*

Small monomorphic trypanosomes with a scanty undulating membrane, and without a free portion to the flagellum, were present in forty-two (8.4 per cent.) of the animals examined, namely, in twenty-eight hump-backed cattle, six straight-backed cattle, two sheep, five pigs, and one goat. The infections were never very heavy, and in several of the hosts only one or two individuals were found in a large blood-film.

Both a guinea-pig and a white rat were on two occasions inoculated with blood containing these small trypanosomes, but with negative results. It was unfortunately impossible to experiment with larger animals, such as goats, sheep, and cattle, and no dogs were available. No detailed measurements at known periods of the infection could therefore be made. Fifty individuals, taken as they came, were, however, measured in blood-films from hump-backed cattle and pigs naturally infected with these parasites (see Table V). The fifty from cattle averaged  $12.94\mu$  in length; the longest measuring  $17\mu$  and the shortest  $10\mu$ . The average breadth was  $2.29\mu$ , the broadest being  $3.5\mu$  and the narrowest  $1.25\mu$ . In pigs the average length of the fifty trypanosomes measured was  $12.24\mu$ , the longest being  $16\mu$  and the shortest  $10\mu$ . In breadth they averaged  $1.99\mu$ , and ranged from  $3.25\mu$  to  $1.25\mu$ . The distribution according to length and breadth of the hundred individuals is shown in Charts III and IV.

The identity of these trypanosomes is open to doubt. Similar specimens from horses which were taken to England from Nigeria in 1911 were identified by Bruce as *T. pecorum*, a species which is apparently indistinguishable from *T. nanum*, and which, according to Blacklock and Yorke (1913), is probably identical with *T. congolense*. The trypanosome found in the Gold Coast is morphologically similar to that found in Nigeria, although in both colonies the parasite was found to vary from the type to a considerable extent. In experiments conducted at Eket this variation was found to occur in the same strain when transmitted to different hosts. There seems to be some reason to suppose that *T. pecorum*, *T. nanum*, and *T. congolense* are all strains of the same species of trypanosome; in which case the name *T. congolense* is that properly applicable to it.

With regard to *T. dimorphon*, such uncertainty exists as to the nature of the original strain that it is doubtful if the name should now be employed. The original figures given by Dutton and Todd (1903) of their Gambian Horse Trypanosome show two types of parasite that would certainly to-day be identified in Nigeria or the Gold Coast as *T. congolense* (*T. nanum*) and *T. pecaui* (*T. brucei* of Uganda). An infected horse sent home some years later by

TABLE V.—The measurements in length and breadth of *T. congolense* (*T. nanum*).

Host	Number measured	Length in microns			Distribution according to length in microns												
		Max.	Average	Min.	8	9	10	11	12	13	14	15	16	17	18		
Pigs ... ..	50	16	12.24	10	—	—	2	11	13	9	7	6	2	—	—		
Cattle, humped ...	50	17	12.94	10	—	—	9	2	10	9	11	3	3	3	—		
	100	17	12.59	10	—	—	11	13	23	18	18	9	5	3	—		

Host	Number measured	Breadth in Microns			Distribution according to breadth in microns												
		Max.	Average	Min.	1	1.25	1.5	1.75	2	2.25	2.5	2.75	3	3.25	3.5		
Pigs ... ..	50	3.25	1.99	1.25	—	4	4	12	14	8	3	4	—	1	—		
Cattle, humped ...	50	3.5	2.29	1.25	—	1	3	3	15	5	12	2	6	2	1		
	100	3.5	2.24	1.25	—	5	7	15	29	13	15	6	6	3	1		

Dr. Todd as probably harbouring *T. dimorphon* proved on investigation by Yorke and Blacklock to have a double infection with *T. congolense* and *T. vivax*. It is probable, therefore, that the animals originally examined by Dutton and Todd were infected with all the three types of trypanosome common in West Africa, namely, those referred to here as *T. vivax*, *T. congolense*, and *T. pecaui*. In the sense in which the name is used by Laveran and Mesnil, *T. dimorphon* denotes a polymorphic species of trypano-

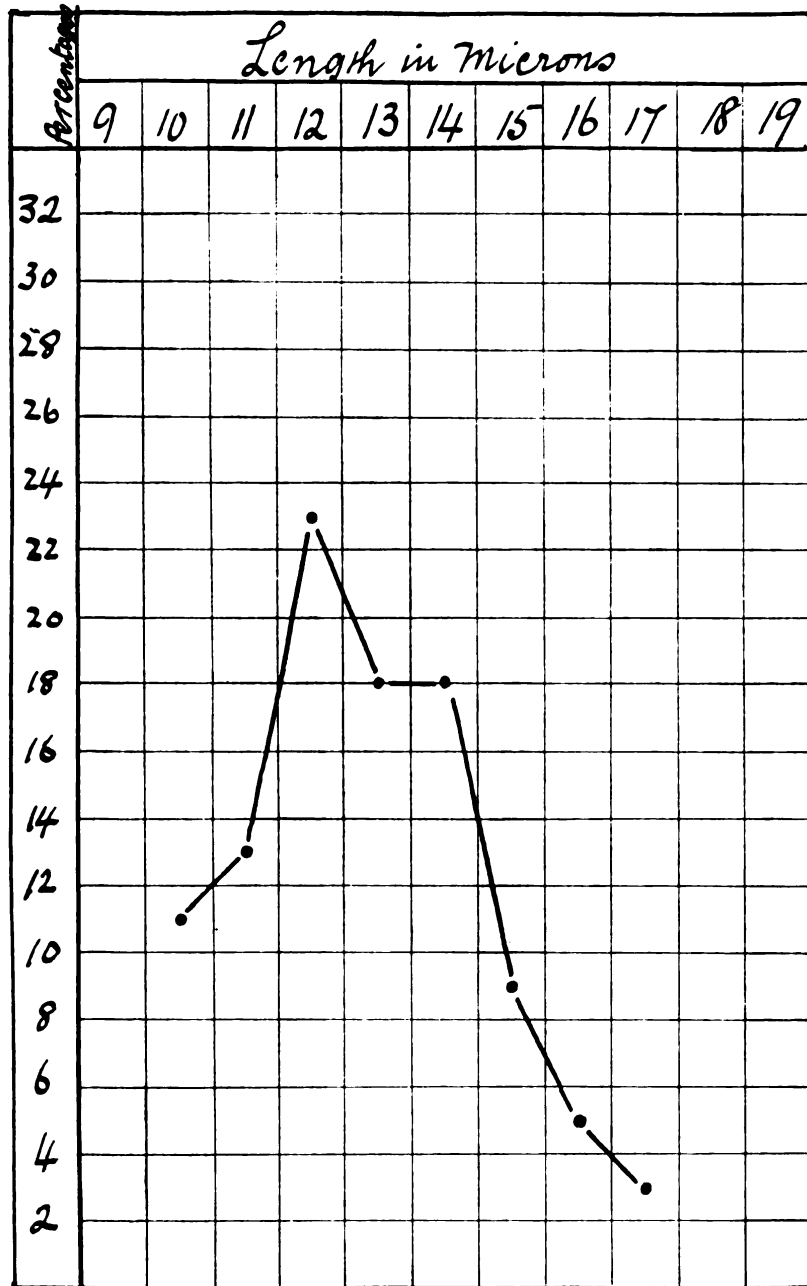


CHART III.—The distribution, by percentages, in length of *T. congolense* (*T. nanum*).

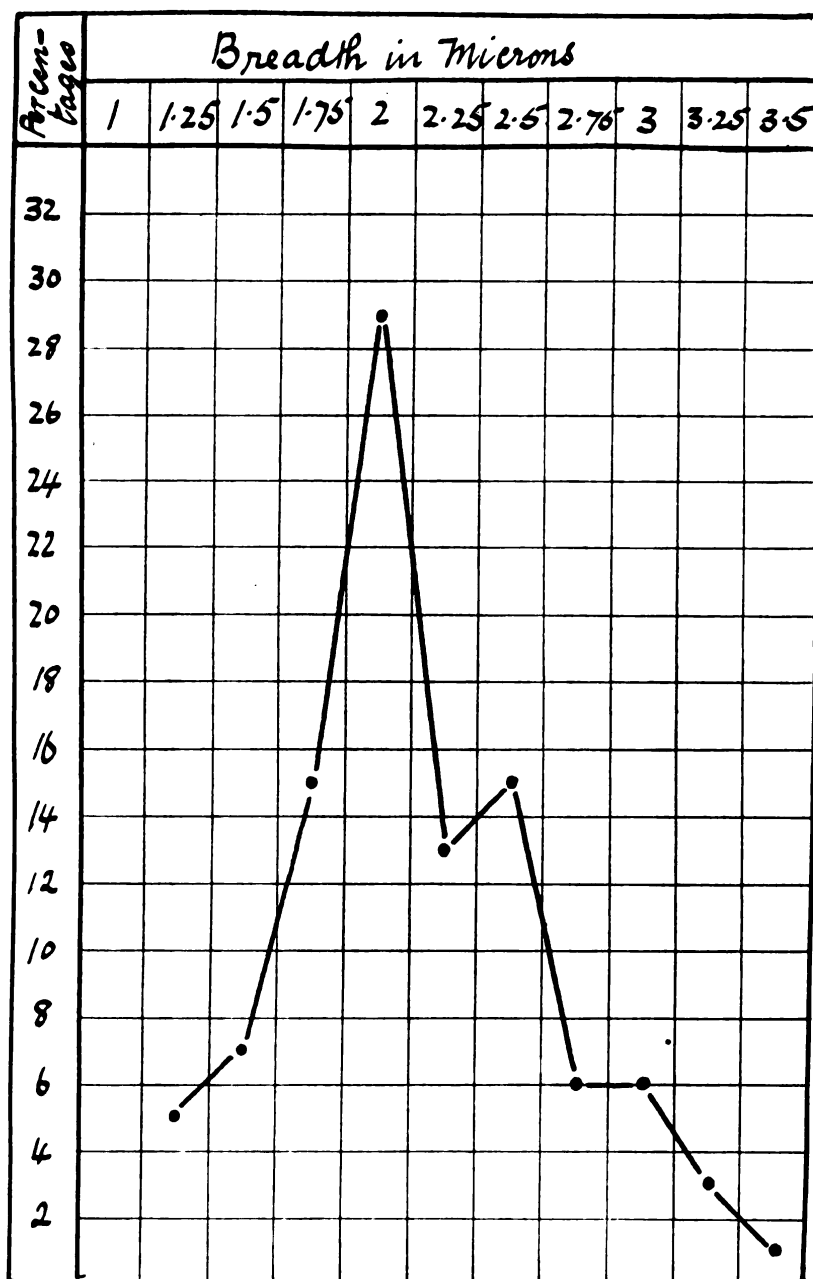


CHART IV.—The distribution, by percentages, in breadth of *T. congolense* (*T. nanum*).

some, highly pathogenic to the smaller laboratory animals, all the individuals of which are without a free flagellum, and which shows forms measuring from  $11\mu$  up to  $30\mu$  in length. Such a trypanosome I have not yet seen in West Africa. Mesnil (1915) considers that the small monomorphic strains of trypanosome isolated by me at Eket from *G. tachinoides* were *T. dimorphon*, apparently because a single individual was found to attain the length of  $21\mu$ . Two strains were isolated by me, and in the second a single trypanosome was measured that reached this length. This strain I noted in my description was unusually large, but I did not separate it off from the other and more typical one because I observed considerable variations in the size of the latter parasite when inoculated into different animals. The trypanosomes of the first and more typical strain measured from  $9\mu$  to  $18\mu$ , and averaged  $13.9\mu$  in length; and I think it could not but have been *T. congolense*. If the second strain were not a variety of the same parasite, as I believe it to have been, it must, I think, have been a new species. Its length, average  $15.15\mu$ , maximum  $21\mu$ , minimum  $11\mu$ , does not approach that of *T. dimorphon*.

The ideal relationship between a parasite and its host would appear to be the benign infection, since a strain that kills its host commits racial suicide. In the case of these trypanosomes an approximation to the ideal relationship exists in game, and in certain domestic animals. It might be expected, however, that a parasite specialised so as to produce a benign infection in a certain type of host might show wide variations in pathogenicity when introduced into other animals. The smaller laboratory animals, such as rats, guinea-pigs, and rabbits, in which the pathogenicity of trypanosomes is generally studied, are species that tsetse flies normally would seldom have an opportunity of infecting, and it might therefore be expected that irregular variations would occur in them as the result of artificial inoculations. The significance of the fact that *T. congolense* is said to differ from *T. nanum* only in the fact that it is pathogenic to these smaller laboratory animals, is therefore of doubtful value.

Strains of trypanosomes that have been carried on for a number of years by inoculations from animal to animal in a laboratory, in unnatural surroundings, often in unnatural hosts, and without the natural alternation of development in the insect (exogenous cycle)



and in the vertebrate (endogenous cycle), might be expected to show abnormalities. One direction in which abnormality might be anticipated would be in the pathogenicity, and it would be most probable that this would take the form of a loss of specificity. This has actually been found to be the case by some observers. Blacklock and Yorke (1913a), for instance, succeeded after a number of passages in infecting rabbits with *T. vivax*, a species of trypanosome that is usually innocuous to these animals. The same observers found that the early inoculations into laboratory animals of a small trypanosome isolated from a horse infected in the Gambia mostly failed, whereas the later were invariably successful.

If such alterations in pathogenicity take place in laboratory strains, it is probable that they also occur occasionally in nature. This might be the result of a natural variation in the virulence of the trypanosome, of some idiosyncrasy of the host, or of both these factors. Some such explanation has indeed been advanced to account for the human infections with polymorphic trypanosomes characterised by posterior nuclear forms (*T. rhodesiense*) which occur in some countries, but apparently not in others in which parasites morphologically identical are common in game and domestic animals.

For the above reasons it seems probable that differences in pathogenicity to the smaller laboratory animals are not of specific importance, although in some localities they may be so constant as to constitute distinct 'races.' And as regards *T. congolense* (*T. pecorum*) and *T. nanum*, that are morphologically identical, that infect the tsetse fly in the same manner, and that differ only in their pathogenicity to laboratory animals, I agree with Blacklock and Yorke (1913b) that in the present state of our knowledge we can only conclude that they are the same parasite.

#### B. CANINE TRYPANOSOMIASIS

Only one dog was examined in whose blood trypanosomes could be found. This animal, a bull-terrier, showed a very small infection with small parasites of the *T. congolense* type. The trypanosomes were too rare for it to be possible to study their morphology by means of measurements; but the few individuals that

were found were similar in shape and size to the trypanosomes of this type found in cattle and horses. The dog was in a very poor condition, much wasted, covered with sores, and with a profuse watery discharge from the eyes. The disease ended fatally.

### C. EQUINE TRYPANOSOMIASIS

Nineteen cases of equine trypanosomiasis were met with in the nine months during which the present investigation was in progress, namely, in thirteen horses, five mules, and one donkey. Seven of the horses were infected with *T. pecaui* (*T. brucei* of Uganda), four with *T. congolense* (*T. pecorum*), and two with *T. vivax* (see Table VI). All the five mules were infected with *T. pecaui* (*T. brucei* of Uganda), and the donkey with *T. congolense* (*T. pecorum*).

TABLE VI.—Equine trypanosomiasis at Accra.

Host	Cases of trypanosomiasis seen	Number infected with		
		<i>T. vivax</i>	<i>T. congolense</i>	<i>T. pecaui</i>
Horses ... ..	13	2	4	7
Mules ... ..	5	—	—	5
Donkeys ... ..	1	—	1	—
Totals ... ..	19	2	5	12

All the animals belonged to Accra, and had not been out of the town for periods varying from two or three months to over ten years. It is practically certain, therefore, that they had been infected actually in Accra itself, either by stray tsetse flies, or by some other biting insect such as a *Stomoxys* or *Lyperosia*.

Mules, which are generally imported from the Canary Islands,

are largely employed in Accra, as they are thought to be less susceptible than horses to trypanosomiasis. The immunity, if it exists, can only be a very partial one, and does not protect them from fatal infections with *T. pecaui*.

The trypanosomes found in horses in Accra were the same as those occurring in cattle, and the remarks already made with regard to the identification of the latter apply also to the former.

One of the horses, however, was infected with a small trypanosome of the *T. congolense* type that differed in some respects from the other parasites of this group found in domestic animals at Accra. A short description of this parasite, and of the clinical features of the disease caused by it, is given below.

*T. pecaui* (*T. brucei* of Uganda) is almost invariably fatal to horses, *T. vivax* almost never so, whilst *T. congolense* (*T. nanum*) has a virulence intermediate between these two. It must be remembered, however, that even *T. vivax* is a serious scourge, since it incapacitates its victims for a very long time.

Trypanosomiasis in Accra should be an easily preventable disease. Tsetse flies do not appear to breed in the immediate vicinity of the town, and the specimens occasionally captured within its bounds are supposed to have been brought in on trains, waggons, motor cars, etc. Occasional cases of trypanosomiasis might therefore be expected, but there would appear to be no reason whatever why there should be so many annually as occur at present. No precautions are taken to prevent the spread of the disease, and animals heavily infected with trypanosomes are allowed to move freely about the town. As, however, it is well known that trypanosomes can be transmitted, at any rate mechanically, by *Stomoxys*, and other blood-sucking insects besides *Glossina*, it would perhaps be vain to expect any reduction in the number of cases in horses and mules in Accra so long as herds of cattle are allowed to loiter about the streets, and to graze wherever they can find a patch of grass. The cattle carry with them wherever they go various biting flies, and as has been shown above, 92 per cent. of the hump-backed cattle and 18 per cent. of the straight-backed breeds are infected with trypanosomes of the same species as those that infect horses and mules.

**D. A SMALL MONOMORPHIC TRYPANOSOME FOUND IN THE  
BLOOD OF A MARE. Pl. XXXVI, figs. 15-28**

One strain of trypanosome of the *T. congolense* group, found in a horse, presented certain morphological features which were unusual, and a short description of this parasite may not be out of place.

The animal in which the infection was found was a mare brought to Accra from England some years ago. She had not been out of Accra for at least eight months before the commencement of her illness, so that it is probable that the infection was contracted in Accra. On the 24th January, 1915, she was sent to the laboratory for examination, because one day she had stumbled and nearly fallen down when being driven, and because it was noticed that her joints were swollen. The first indications of illness had been observed two or three days before this date.

On examination, the mare was found to be in good condition, but there was some oedema of the legs, and in the blood a few trypanosomes were found. During the next few days the trypanosomes increased in number; but on the 5th February they were again scarce. On the 6th February, two localised patches of oedema, each about the size of the palm of the hand, appeared on the left side; the one in the saddle area, and the other a little further back. Three days later these raised patches or plaques had almost entirely vanished.

Trypanosomes continued to be constantly present in the blood, but were never plentiful; and the mare gradually lost condition until, by the end of February, she was obviously extremely ill. On the 4th March she became suddenly much worse: the oedema of the legs became considerable, the anaemia very marked, the hind feet began to drag badly, some discharge from the eyes appeared, and all over the body there appeared a large number of little raised patches of oedema, each about the size of a shilling or a little larger. These patches or plaques could be distinctly felt as raised discs with a more or less circular outline, and a definite edge. The hair over them was slightly roughened. The trypanosomes were also more numerous in the blood, but they were still not abundant.

Up to this time treatment with injections of Atoxyl, and

arsenious acid and perchloride of mercury by the mouth had been employed; but as these drugs had not produced any perceptible benefit, on the 8th March, at the request of the owner, a native horse-doctor was given the chance of proving the efficacy of his methods. On the 21st March, however, it was reported that the 'cure' had failed, and that the mare was much worse. I saw her the following day, and examined her blood, and found trypanosomes still present, but not numerous. She was then obviously dying, and was unable to leave her stable. She was greatly wasted, her skin was covered with small patches of oedema or plaques, the oedema of the legs and abdomen had increased greatly, there was profuse diarrhoea, and discharges were running from the eyes, nose, and vagina. On the following afternoon, March 23rd, the mare died, the disease having lasted just two months.

#### *The Morphology of the Parasite*

The living, unstained trypanosomes were short, and not notably active organisms. They did not show any marked tendency to progress, but, like so many of the trypanosomes belonging to this group, were often seen buried between clumps of red blood corpuscles.

When fixed and stained the parasites were seen to be short, rather stumpy, monomorphic trypanosomes. They were studied in films stained by Leishman's method, and their general appearance is shown in Plate XXXVI. The ratio of breadth to length was usually 1 to 6.8. The posterior end was pointed, or sub-acute. The anterior end tapered rapidly in front of the macronucleus, somewhat in the manner characteristic of *T. vivax*. The micronucleus was small, occasionally terminal, but more usually lying some distance from the pointed posterior end, and sometimes situated laterally. The macronucleus was oval, and was frequently situated at the extreme anterior end of the body (Pl. XXXVI, figs. 18, 21, and 22). On some days this remarkable position of the macronucleus was observed in 50 per cent. of the trypanosomes. The undulating membrane was poorly developed, and was, indeed, seldom visible at all. The flagellum was without any free portion. The cell contents were sometimes homogeneous or showing a finely reticulated structure, and sometimes contained a few granules posterior to the macronucleus. In the final stages of the disease, many of the

trypanosomes showed a single deeply stained chromatin dot immediately posterior to the macronucleus. This body closely resembled a second, and larger, micronucleus in its appearance and in its reactions to stains, but the parasites in which it was seen were not dividing forms. This feature was not peculiar to this strain. Similar bodies have been seen in other trypanosome infections. But as they were a most conspicuous feature in this case, and as they appear in the figures, they should be mentioned.

Measurements were made of the length of three hundred trypanosomes (see Table VII and Chart V), fifty being taken as they came

TABLE VII.—Measurements in length of a small monomorphic trypanosome found in a mare.

Date	Number counted	Length in microns			Distribution according to length in microns												
		Max.	Average	Min.	8	9	10	11	12	13	14	15	16	17			
January 24 ... ..	50	16	12.78	10	—	—	3	7	14	10	7	8	1	—			
February 23 ... ..	50	15	12.10	9	—	1	5	8	18	12	4	2	—	—			
February 26 ... ..	50	15	11.98	8	1	2	4	12	12	11	6	2	—	—			
March 4 ... ..	50	16	12.98	8	1	1	3	3	8	14	11	7	2	—			
March 8 ... ..	50	16	12.66	9	—	1	5	3	13	15	6	6	1	—			
March 22 ... ..	50	17	12.64	8	1	—	8	5	9	9	9	7	1	1			
	300	17	12.52	8	3	5	28	38	74	71	43	32	5	1			

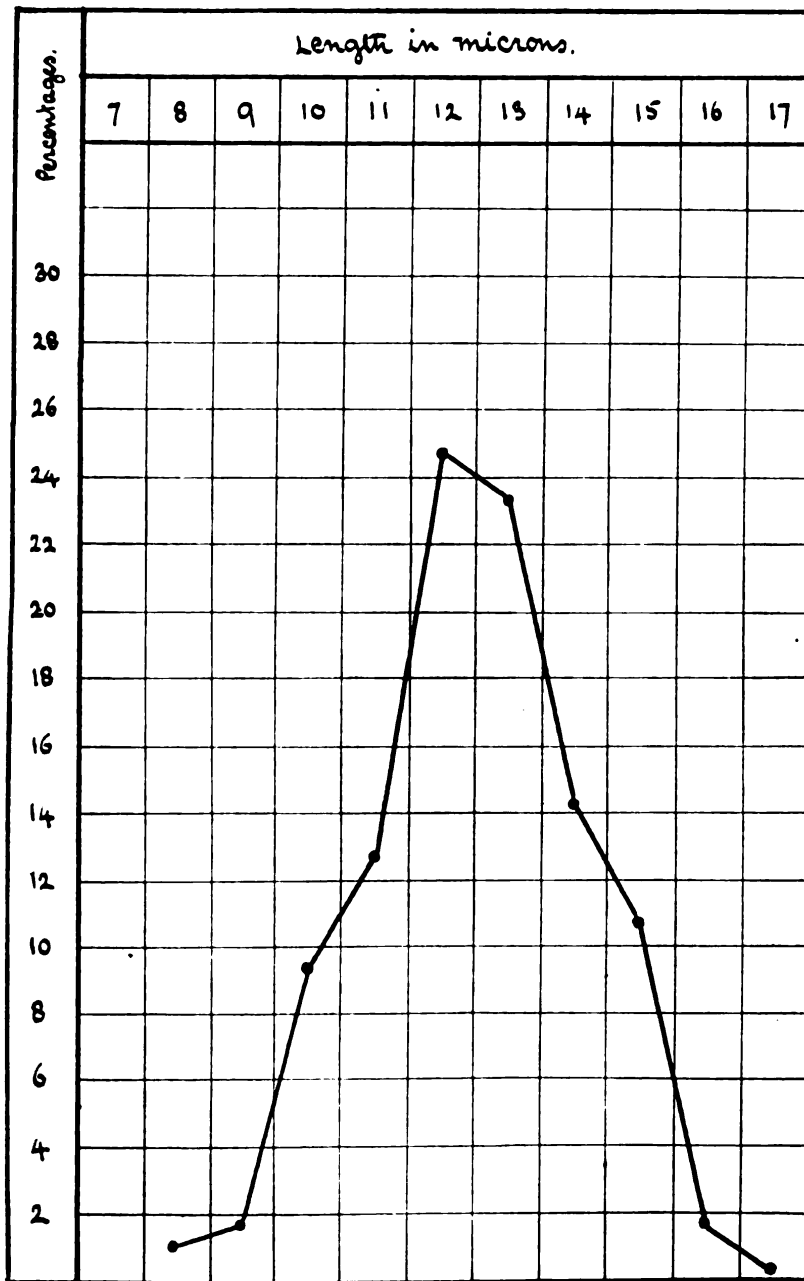


CHART V.—Distribution, by percentages, in length of a small monomorphic trypanosome found in a mare.

on each of the six days on which it was possible to find this number in the blood-films. The average length was  $12.52\mu$ , the longest individual measuring  $17\mu$ , and the shortest  $8\mu$ . The breadth at the widest point was measured in 275 of the same trypanosomes. The average was  $1.84\mu$ , the broadest measuring  $3.5\mu$ , and the narrowest  $1.0\mu$  (see Table VIII, and Chart VI).

TABLE VIII.—Measurements in breadth of a small monomorphic trypanosome found in a mare.

Date	Number counted	Breadth in microns			Distribution according to breadth in microns												
		Max.	Average	Min.	1	1.25	1.5	1.75	2	2.25	2.5	2.75	3	3.25	3.5		
January 24 ...	50	3.0	1.83	1.0	2	2	13	9	14	6	3	—	1	—	—		
February 23 ...	25	3.0	2.15	1.25	—	1	2	3	5	4	3	3	4	—	—		
February 26 ...	50	3.0	1.80	1.0	2	4	11	9	18	4	—	1	1	—	—		
March 4 ...	50	3.5	1.85	1.25	—	7	9	6	21	4	1	1	—	—	1		
March 8 ...	50	2.75	1.87	1.25	—	4	9	9	19	5	3	1	—	—	—		
March 22 ...	50	3.0	1.73	1.0	6	6	12	6	11	3	4	1	1	—	—		
	275	3.5	1.84	1.0	10	24	56	42	88	26	14	7	7	—	1		

#### *The susceptibility of experimental animals*

Owing to the scarcity of animals, inoculations with this trypanosome could only be made into three white rats, two guinea-pigs, and one rabbit. None of these animals became infected.

#### *Identification*

The morphology and the measurements of this trypanosome clearly show its affinity to the species *T. congolense sensu lato*; that is, the *T. pecorum* of Bruce, the synonyms for which, he says, are *T. confusum* (Kinghorn and Montgomery) and *T. nanum* (Laveran), and to which *T. dimorphon* (Laveran and Mesnil) and *T. congolense* (Brodin) are closely allied species. In consequence of the failure to infect rats, guinea-pigs, and a rabbit in the few attempts made, it would probably be considered as most nearly



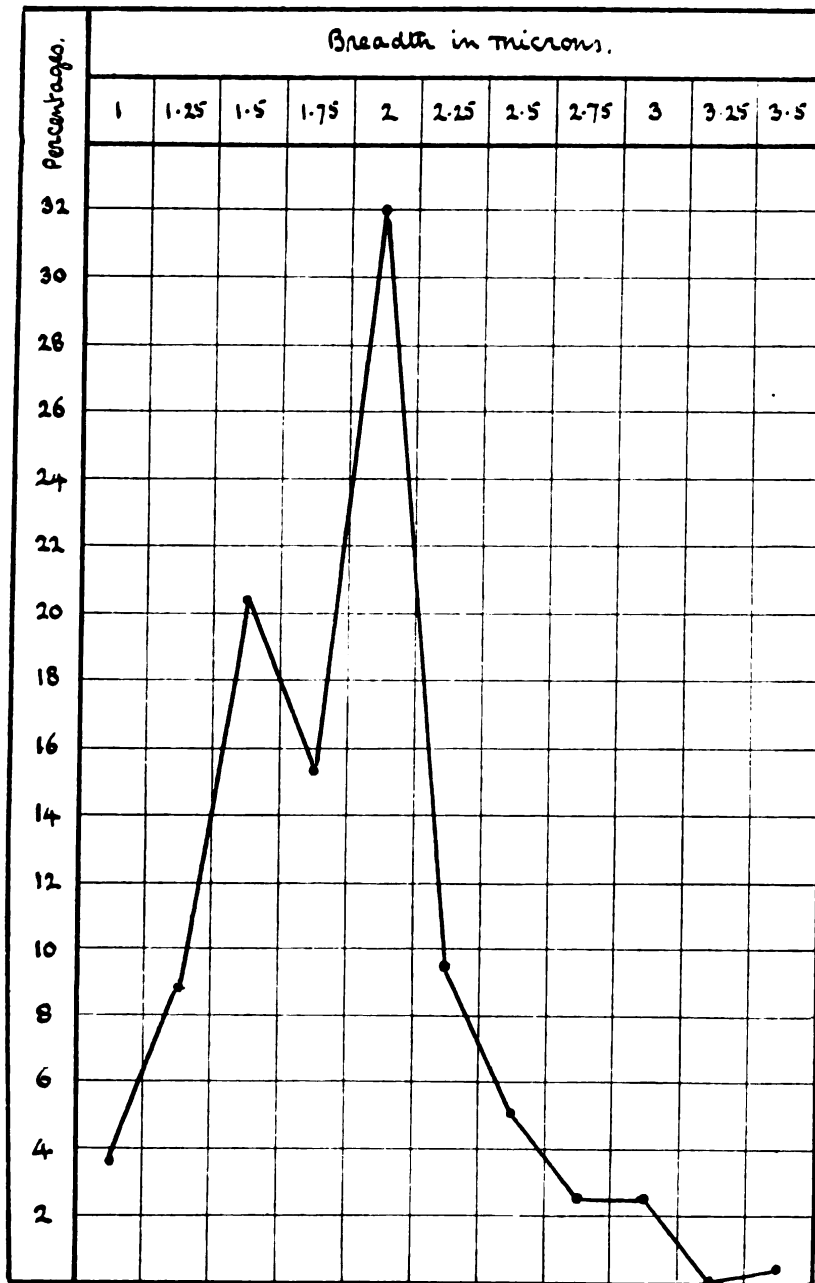


CHART VI.—Distribution, by percentages, in breadth of a small monomorphic trypanosome found in a mare.

allied to *T. nanum* by those who adhere to the view that this species is valid.

The morphology of the parasite, however, showed certain peculiarities. The average length,  $12.52\mu$ , is considerably less than that given for either *T. nanum* or *T. pecorum*, although the trypanosomes were studied in thin blood-films that had been allowed to dry, a procedure which is said to tend to increase their dimensions. Bruce (1914) gives the average length of *T. pecorum* as  $14\mu$ , and in an earlier paper (1911) as  $13.3\mu$ ; and Kinghorn and Yorke (1913) give the figure  $13.6\mu$ . The average length of the monomorphic trypanosomes of this type which I isolated from *G. tachinoides* at Eket in Nigeria (1914) was also considerably greater, namely  $14.1\mu$ . The range of length,  $8\mu$  to  $17\mu$ , is, however, very much the same as that given by most authors for trypanosomes of this species (*T. congolense* in its wide sense); and this is probably of greater importance than the average, since the latter may be subject to variations from a number of causes, and certainly shows marked differences, even in the same strain, when inoculated into different animals.

The peculiar position of the macronucleus, at the extreme anterior end of the parasite, was a most conspicuous characteristic of the trypanosome, and could not, I think, have been due to the technique employed in making the blood-films. I have not been able to find any reference to this peculiarity in the descriptions of any other similar trypanosomes. The occurrence of these forms would seem to mark off this trypanosome as at any rate a well-defined variety. The clinical aspect of the disease in the original host was also unusual. The appearance of raised disc-like patches or plaques on the skin was peculiar in my experience in West Africa. For convenience in reference I suggest, therefore, that this trypanosome should be named *Trypanosoma congolense*, var. *equinum*.

#### E. TWO CASES OF TRYPANOSOMIASIS IN MULES RESEMBLING ACUTE DOURINE

Quite recently, two mules have come under my notice suffering from an infection with a polymorphic trypanosome of the type *T. pecaudi* (*T. brucei* of Uganda). In both these animals the

remarkable features were the rapidity with which the disease developed, and the appearance shortly before death of small patches of oedema on the skin.

The first of the two mules (see Pl. XXXV, fig. 1) was brought to me on the afternoon of June 16th. It was hardly able to move, the hind legs being stiff and paresed; the head had to be supported or the animal would have fallen, the eyes were closed, and there was a watery discharge from the eyes and the nostrils. Oedema was not conspicuous, but the legs were a little puffy. Trypanosomes were found in the blood, but they were very scanty. The animal was somewhat wasted, and looked as if it were in the very last stages of trypanosomiasis; and it was hardly credible that it could have been at work and apparently in its normal health the same morning, which nevertheless was the case. The next day the mule was even worse, and stood in the stable with its head bent almost to the ground, and resting against the wall, its hind quarters drooping, and the right hind hoof turned backwards from the fetlock. On this day, all over the skin of the neck and sides a number of small plaques were observed, each about the size of a shilling; and the general oedema began to increase especially in the dependent parts of the face. On June 19th, the third day after the onset of the symptoms, the animal was in such a distressing condition that it had to be destroyed.

The second mule was first seen on June 17th. In the morning it had been apparently quite well, and had done its usual round of work. At 3 p.m., when I saw it, it was in very much the same condition as that described above in the case of the first mule. The gait in particular was remarkable, and suggested irresistably that the animal was extremely drunk. The plaques on the skin (see Pl. XXXV, fig. 2) were first seen on June 18th, and on the 19th the animal fell down, and had to be killed, as it was unable to rise again.

In neither of these animals were the trypanosomes numerous in the blood, but sufficient were examined to prove that they were morphologically identical with the type of parasite found in cattle and horses in Nigeria and the Gold Coast, and which is referred to in this paper as *T. pecaudi*.

Owing to the lack of experimental animals, it was only possible to inoculate a single guinea-pig from each of the infected mules.

The guinea-pig inoculated from the first mule did not become infected. That inoculated from the second mule first showed trypanosomes in the blood on the seventh day, and died on the ninth day. Another guinea-pig inoculated from it died two days later from some unknown cause, and as the mule itself had died in the meantime, the strain was unfortunately lost. In the blood of the single guinea-pig that became infected, the trypanosomes were numerous on the last two days, and forms with posteriorly placed nuclei were common. The measurements in length of this trypanosome in the one mule in which it was possible to find twenty-five parasites in the slides taken, and in the guinea-pig infected from it, are shown in Tables IX and X. The average length was  $22.39\mu$ , the longest being  $33\mu$ , and the shortest  $13\mu$ .

The rapidity with which the symptoms developed in these two cases was unique in my experience. The course of the disease caused by the polymorphic trypanosome commonly found in Nigeria and the Gold Coast (*T. pecaui*), although usually rapid, has always been a matter of weeks or months in the horses and donkeys that I have examined. The onset has always been rather gradual, the first indications of illness being a loss of vitality, and a tendency to stumble, and the symptoms then slowly developing, some cases terminating fatally in two to four weeks, others lingering on for as many months. Laveran and Mesnil (1912) state that the duration

TABLE IX.—Measurements of 75 trypanosomes of the polymorphic strain found in two mules at Accra.

Host	Number measured	Day of the infection	Length in microns		
			Average	Minimum	Maximum
Mule No. 923 ...	25	Natural infection. Two days before death	20.76	13	32
Guinea-pig No. 79 ...	25	Inoculated from Mule No. 923. First day of infection	25.14	17	32
Guinea-pig No. 79 ...	25	Inoculated from Mule No. 923. Second day of infection, and day before death	21.28	16	33
			22.39	13	33

TABLE X.—Distribution according to length of 75 trypanosomes of the polymorphic strain found in two mules at Accra.

Host	Number measured	Day of infection	Length in microns										
			13	14	15	16	17	18	19	20	21	22	23
Mule No. 923 ...	25	Natural infection. Two days before death	1	1	3	2	—	3	2	—	1	2	1
Guinea-pig No. 79 ...	25	Inoculated from Mule No. 923. First day of infection	—	—	—	—	1	1	1	3	1	1	1
Guinea-pig No. 79 ...	25	Inoculated from Mule No. 923. Second day of infection, and day before death	—	—	—	1	3	4	4	3	2	2	—
Totals ... ..			1	1	3	3	4	8	7	6	4	5	2

Host	Number measured	Day of the infection	Length in microns									
			24	25	26	27	28	29	30	31	32	33
Mule No. 923    ...    ...	25	Natural infection. Two days before death	3	2	1	1	1	—	—	—	1	—
Guinea-pig No. 79    ...	25	Inoculated from Mule No. 923. First day of infection	2	2	1	2	—	2	3	3	1	—
Guinea-pig No. 79    ...	25	Inoculated from Mule No. 923. Second day of infection, and day before death	—	—	1	2	—	1	—	1	—	1
Totals    ...    ...    ...			5	4	3	5	1	3	3	4	2	1

of the disease due to *T. pecaudi* is three to four months. In no other cases have I seen such a dramatic onset as occurred in these two mules, which were apparently well in the morning, and obviously dying the same afternoon.

Neither have I previously seen the little patches of oedema of the skin in infections with polymorphic trypanosomes in West Africa. These patches were raised, and rounded, and about the size of a shilling or a little larger. Over them the hair bristled (see Pl. XXXV, fig. 2). They answered very closely to the description given by Pease of the plaques in dourine, as looking as though a metal disc had been slipped under the skin. Such plaques are generally considered to be characteristic of dourine (*T. equiperdum*), and indeed the general appearance of the animals, and their attitude, which almost exactly resembled that shown by Laveran and Mesnil (1912) in their fig. LXXIII, suggested this disease. Cazalbou, however, has described similar cutaneous lesions in infections with *T. pecaudi*, but Bouffard did not observe them, and Pécaud considered that they were rare. In another part of this paper I have described the case of a mare infected with trypanosomes of the *T. congolense* type, in which similar dermal plaques were a prominent symptom. Plaques are not therefore pathognomonic of dourine, although they must still remain an important sign for the purposes of differential diagnosis.

Dourine does occur in an acute form, as described by Laveran and Mesnil, in which 'à l'engorgement du début, succèdent une paralysie aiguë soudaine ou des accès de vertige qui emportent le malade en quelques jours'; and this account corresponds with that given above of the course of the disease in these two mules. The morphology of the trypanosome found in these cases was moreover not incompatible with a diagnosis of dourine, since of the four strains of *T. equiperdum* examined by Blacklock and Yorke (1913) one, for which they proposed the name *T. equi*, was found to be dimorphic, and indistinguishable from *T. rhodesiense*.

After most careful enquiries, however, it was proved conclusively that neither of the mules could possibly have contracted the disease in coitus. Both were mares, and had been in Accra for five and three years respectively, during which time they had not been covered. Unless, therefore, *T. equiperdum* (*T. equi*) can also be conveyed by some other means, such as the bites of insects, which is

not altogether impossible, these mules cannot have been infected with this species of trypanosome.

So far as I am aware, dourine (Mal du Coït) is not known to occur in the Gold Coast. I am convinced, nevertheless, that the infection in these two mules cannot have been the same as that due to the polymorphic trypanosome of which I had the opportunity of studying a large number of cases in horses in Nigeria. I am inclined to think that the trypanosome may have been the same as the Runcorn Laboratory strain of *T. equiperdum* described by Blacklock and Yorke (1913), which was one that had been brought from Algiers in a horse by the firm of Hagenbeck. This was the dimorphic strain indistinguishable from *T. rhodesiense*, for which the name *T. equi* was proposed. In the present uncertainty as to the identity of mammalian trypanosomes, it is impossible to determine what is the connexion between this dimorphic *T. equiperdum* and the monomorphic strains which are generally known by that name. It is equally impossible to determine whether or not, in a country in which tsetse flies exist, the trypanosomes causing the disease clinically known as dourine may not be capable of transmission by these insects as well as mechanically in coitus. It is, however, of interest to record the occurrence of a disease clinically resembling acute dourine in mules in whom infection by coitus may be excluded with certainty.

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EXPLANATION OF PLATES

PLATE XXXV

Trypanosomiasis of mules resembling acute dourine.

Fig. 1. Showing the advanced stage to which the disease had progressed within twenty-four hours of the onset of the symptoms.

Fig. 2. Showing the plaques on the skin.





FIG. 1

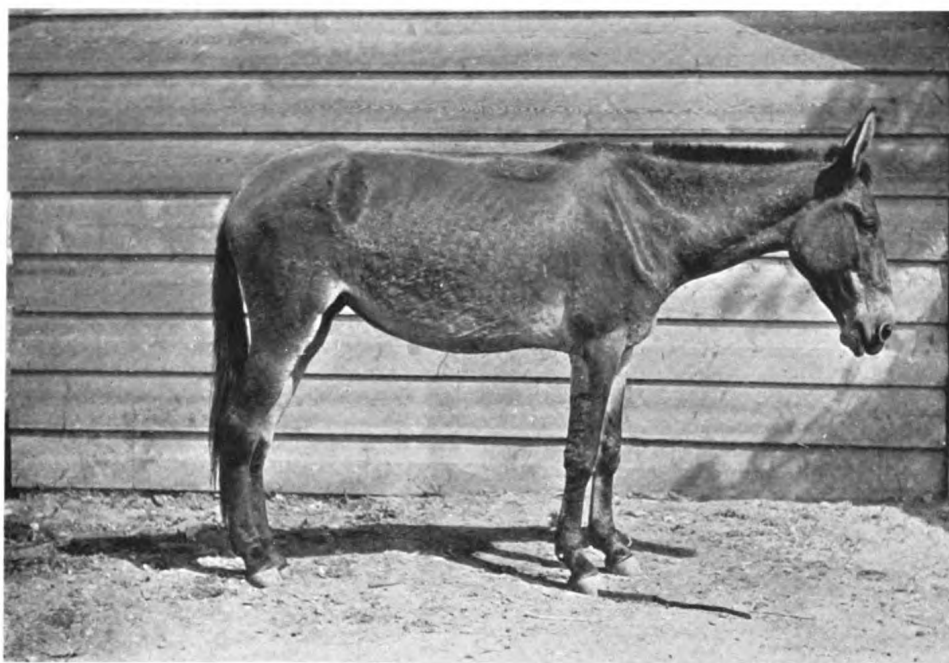


FIG. 2





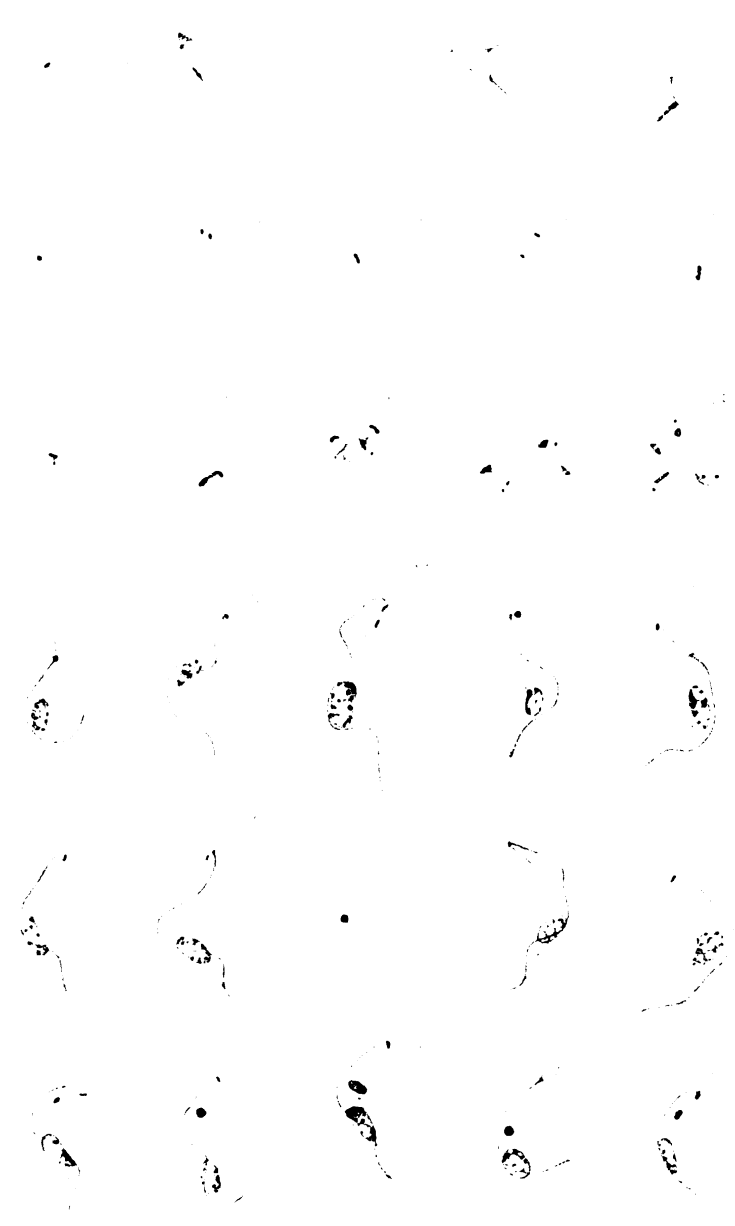
## PLATE XXXVI

Figs. 1-14. A piroplasm of the brown rat—*Nuttallia decumani*,  
n. sp. × 2000.

Figs. 15-28. A small monomorphic trypanosome found in the  
blood of a mare—*T. congolense*, var. *equinum*. × 2000.

Fig. 29. A red blood corpuscle containing an *Anaplasma*-like body  
such as became very common in the blood of this mare in  
the latter stages of the disease. × 2000.

1890





# DIFFERENTIAL COUNTS AND THE NEUTROPHILE BLOOD PICTURE OF NATIVES—ADULTS AND CHILDREN— OF NEW GUINEA

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## INTRODUCTION

Our knowledge as to the blood conditions of coloured native races is comparatively limited. It is only within the last few years that Chamberlain and Vedder (1911) recorded observations on the blood of Filipinos, Bahr (1912) on that of Fijians, and Marshall and Meerwein (1913) on 'wild' natives of German New Guinea.

The Arneth blood picture has been studied in Filipinos by Chamberlain and Vedder, and in New Guinea natives by Marshall and Meerwein. Unfortunately only the observations on Filipinos can be used for comparison, as our results, as well as those of Chamberlain and Vedder, were obtained by the use of the original Arneth classification, whilst Marshall and Meerwein employed a somewhat different classification.

Chamberlain and Vedder pointed out that the Arneth index in natives (Filipinos) showed a distinct increase when compared with that of normal Europeans.

Our observations on the Arneth index of white school children, born and reared in the tropics (North Queensland), showed a change in the same direction.

As opportunity offered, it was deemed of interest to investigate the neutrophile blood picture of New Guinea natives, as well as the percentages of the different types of white blood corpuscles, adults and children being considered separately.

The material was collected during a journey through the coastal parts of British New Guinea, blood films being made from natives of different ages. Some selection was made in the case of adults, blood films being obtained only from apparently healthy natives who lived in comparatively clean villages, and all blood films containing microfilariae or malarial parasites were discarded. As the majority of the blood films were made during the day, infections with microfilariae possessing a turnus could not be excluded. The blood films were taken from natives who had been in contact with Europeans for a considerable period, as well as from inhabitants of villages which had not come under Government control, but no striking differences could be observed.

With children it was practically impossible to make a similar selection because of the wide distribution of yaws and malaria, although children with apparent manifestations of yaws and skin diseases were excluded, and films containing malarial parasites were not considered in the present investigation.

The blood films were stained after Giemsa's method, and for the differential and Arneth counts the same technique was adopted as employed in our blood examination of North Queensland school children.

The results are based on the examination of 104 films from adults and 50 from children, the latter, as far as one could judge, were below 10 years of age.

#### DIFFERENTIAL COUNTS

For the differential counts 500 successive leucocytes were enumerated.

Considerable variations were observed in the percentages of the different types of leucocytes, if individual counts be considered (see Table I). The average figure for the polymorphonuclear leucocytes in adults was 51.06 per cent., and the range lay between 28.9 per cent. and 81 per cent. This figure is somewhat below the normal European average (55 to 70 per cent.) and is lower than the average figure obtained for North Queensland school children (51.1 per cent.).



TABLE I.

Differential and Arneth Counts on the Blood of 104 Adult Natives from New Guinea.

Differential Counts per cent.						Arneth Classification per cent.					Arneth Index
Number	Polymorpho- nuclear	Transitional	Large Mononuclear	Lymphocytes	Eosinophiles	I	II	III	IV	V	
1	71.2	4.6	1.4	13.4	9.4	42.5	38.0	17.0	2.5	—	
2	61.6	3.6	3.4	28.8	2.6	32.5	42.5	19.5	5.5	—	
3	61.4	3.2	2.2	25.6	7.6	40.0	46.0	12.5	1.5	—	
4	63.0	2.8	2.8	25.6	5.8	26.0	46.5	21.0	6.5	—	
5	52.6	6.4	3.6	28.6	8.8	34.0	50.0	12.5	2.5	1.0	
6	48.0	7.6	3.0	33.0	8.4	40.0	47.5	12.0	0.5	—	
7	57.8	4.6	0.2	21.6	15.8	17.0	38.0	35.0	9.0	1.0	
8	59.6	6.0	—	25.2	9.2	15.0	41.0	30.0	10.0	4.0	
9	58.8	6.0	2.0	26.8	6.4	33.5	41.0	20.0	5.0	0.5	
10	39.6	4.4	5.6	36.8	13.6	34.0	42.5	20.0	3.5	—	
11	56.8	7.6	3.0	27.4	5.2	34.5	44.0	17.5	3.5	0.5	
12	35.0	3.3	1.4	29.3	31.0	34.0	41.0	21.0	3.5	0.5	
13	60.8	7.4	2.4	16.8	12.6	23.0	44.0	26.5	6.0	0.5	
14	62.6	3.8	2.0	18.0	13.6	26.5	47.5	23.5	2.5	—	
15	49.0	5.4	0.8	31.8	13.0	45.0	41.5	10.5	3.0	—	
16	68.0	2.6	3.0	17.4	9.0	31.0	43.0	22.0	4.0	—	
7	60.0	2.4	0.8	18.8	18.0	25.0	46.0	21.5	6.0	1.5	
8	64.0	2.8	1.0	17.4	14.8	28.0	40.5	25.5	5.5	0.5	
9	40.0	4.8	2.8	29.2	23.2	26.0	38.5	26.5	8.0	1.0	
0	59.8	2.0	3.0	15.2	20.0	20.0	46.0	26.0	7.0	1.0	
1	46.4	3.6	3.2	30.6	16.2	39.5	41.5	13.5	4.5	1.0	
2	43.2	4.6	2.0	37.4	12.8	40.0	46.5	11.0	2.5	—	
3	32.8	5.6	1.4	33.4	26.8	36.5	44.0	17.5	2.0	—	
4	39.3	5.0	1.5	27.2	27.0	39.0	46.5	12.0	2.5	—	
5	76.0	3.2	1.0	15.0	4.8	30.0	45.0	21.0	3.5	0.5	
6	60.8	1.6	2.4	25.2	10.0	22.5	42.5	25.5	9.5	—	

TABLE I.—Continued.

Number	Differential Counts per cent.					Arneith Classification per cent.					Arneith Index
	Polymorpho- nuclear	Transitional	Large Mononuclear	Lymphocytes	Eosinophiles	I	II	III	IV	V	
27	68.6	4.0	1.0	23.6	2.8	54.5	36.0	8.0	1.5	—	
28	53.6	2.8	0.6	33.8	9.2	37.0	43.5	15.0	3.5	1.0	
29	52.2	5.5	3.0	26.8	12.5	27.5	48.0	19.5	4.5	0.5	
30	62.7	3.3	1.5	22.0	10.5	40.0	44.5	13.0	2.0	0.5	
31	56.0	2.4	1.0	27.4	13.2	33.0	45.5	18.5	3.0	—	
32	63.4	3.6	1.0	23.6	8.4	33.0	39.0	22.5	5.5	—	
33	57.8	2.4	1.6	30.2	8.0	28.5	37.0	26.0	7.0	1.5	
34	53.0	3.4	0.8	25.0	17.8	21.5	38.5	30.0	10.0	—	
35	63.0	2.8	0.2	23.0	11.0	22.5	46.5	20.0	10.5	0.5	
36	46.0	4.2	1.4	38.8	9.6	29.5	37.5	23.0	9.0	1.0	
37	41.0	2.6	1.6	42.8	12.0	40.5	40.0	17.0	2.0	0.5	
38	74.0	2.8	1.0	15.4	6.8	20.5	39.0	29.5	10.0	1.0	
39	51.4	4.8	1.6	37.8	4.4	57.0	37.0	5.0	1.0	—	
40	41.6	2.0	1.0	38.2	17.2	41.0	41.0	17.0	1.0	—	
41	50.2	2.0	3.0	37.0	7.8	53.0	37.0	8.5	1.5	—	
42	41.4	1.4	0.6	42.0	14.6	47.5	37.5	12.5	2.5	—	
43	40.0	1.8	1.2	51.0	6.0	35.0	46.0	16.0	2.0	1.0	
44	81.0	1.2	0.6	16.8	0.4	39.0	42.0	16.0	3.0	—	
45	40.0	2.0	1.4	27.0	29.6	49.0	42.5	8.0	0.5	—	
46	60.2	2.8	1.4	25.0	10.6	35.5	50.5	11.5	2.0	0.5	
47	59.0	1.4	1.2	30.8	7.6	21.0	41.5	26.0	11.0	0.5	
48	43.6	3.4	1.8	38.4	12.8	29.0	50.5	16.0	4.5	—	
49	66.2	2.0	0.4	24.8	6.6	39.5	40.0	18.0	2.0	0.5	
50	51.0	1.6	1.0	37.6	8.8	40.5	43.0	12.5	3.5	0.5	
51	44.0	1.2	0.8	37.0	17.0	25.0	47.5	21.0	4.0	2.5	
52	39.6	4.0	2.4	44.0	10.0	41.0	43.0	12.0	4.0	—	

TABLE I.—Continued.

Number	Differential Counts per cent.					Arneth Classification per cent.					Arneth Index
	Polymorpho- nuclear	Transitional	Large Mononuclear	Lymphocytes	Eosinophiles	I	II	III	IV	V	
53	50.4	1.6	1.0	34.0	13.0	37.5	36.5	22.5	3.0	0.5	
54	61.2	2.4	1.2	27.0	8.2	35.0	39.0	21.5	4.5	—	
55	41.6	4.4	3.0	41.4	9.6	38.5	50.5	10.0	1.0	—	
56	28.9	2.6	1.6	47.8	19.1	42.5	43.0	13.5	1.0	—	
57	45.8	1.6	2.4	40.8	9.4	20.0	49.0	24.5	5.5	1.0	
58	45.0	2.4	2.0	35.6	15.0	14.5	41.0	29.5	12.5	2.5	
59	49.4	2.4	2.0	32.6	13.6	26.5	47.0	24.0	2.5	—	
60	60.4	1.0	1.4	19.4	17.8	22.5	47.0	26.5	4.0	—	
61	49.4	2.2	1.2	33.2	14.0	37.5	45.5	14.5	2.0	0.5	
62	48.4	2.4	2.2	38.2	8.8	27.5	46.0	22.0	4.0	0.5	
63	47.2	1.8	2.0	34.6	14.4	27.0	41.0	29.0	3.0	—	
64	52.2	1.2	0.8	34.4	11.4	27.5	47.0	23.5	1.5	0.5	
65	56.2	2.0	2.8	23.2	15.8	32.5	45.5	20.5	1.5	—	
66	44.4	1.4	1.8	40.6	11.8	18.5	42.0	28.5	10.0	1.0	
67	72.6	2.4	1.8	21.4	1.8	33.5	51.5	13.5	1.5	—	
68	56.8	2.4	1.0	27.0	12.8	18.5	46.5	24.5	9.5	1.0	
69	39.2	2.5	1.6	36.5	20.2	24.0	44.5	28.0	3.5	—	
70	48.6	2.0	0.6	32.2	16.6	27.5	48.0	21.5	3.0	—	
71	48.0	1.2	2.2	41.2	7.4	24.0	47.0	24.5	3.5	1.0	
72	47.2	2.6	1.2	28.2	20.8	18.0	43.5	31.5	7.0	—	
73	61.0	1.4	2.8	26.0	8.8	17.5	44.5	31.5	6.0	0.5	
74	51.6	1.8	2.2	34.8	9.6	19.5	46.0	30.0	4.5	—	
75	55.0	2.6	1.6	29.4	11.4	15.0	45.0	30.0	9.0	1.0	
76	46.2	2.0	2.2	38.6	11.0	19.0	45.5	30.0	5.0	0.5	
77	45.0	2.8	2.2	40.4	9.6	33.5	44.0	19.5	2.5	0.5	
78	56.2	2.2	2.4	32.8	6.4	34.0	46.5	17.5	2.0	—	

TABLE I.—Continued.

Differential Counts per cent.						Armeth Classification per cent.					
Number	Polymorpho- nuclear	Transitional	Large Mononuclear	Lymphocytes	Eosinophiles	I	II	III	IV	V	Armeth Index
79	48.8	1.2	1.4	44.0	4.6	35.0	40.0	20.0	5.0	—	
80	39.0	2.1	2.1	46.2	10.6	29.0	41.5	24.5	5.0	—	
81	46.2	2.8	6.6	40.4	4.0	49.5	37.0	12.5	1.0	—	
82	53.4	3.8	2.4	36.0	4.4	30.5	46.5	19.0	4.0	—	
83	44.0	3.6	1.8	40.0	10.6	44.5	46.0	9.5	—	—	
84	43.8	2.4	1.6	38.6	13.6	18.0	42.5	30.5	8.0	1.0	
85	43.0	4.2	3.4	37.4	12.0	18.0	45.5	30.0	5.5	1.0	
86	46.6	1.8	1.6	44.4	5.6	34.0	39.5	22.5	4.0	—	
87	44.6	2.2	1.2	36.8	15.2	24.0	49.5	21.5	4.0	1.0	
88	51.2	1.4	0.8	27.4	19.2	25.5	47.0	25.0	2.5	—	
89	30.0	2.8	1.2	45.4	20.6	31.0	45.0	20.5	3.5	—	
90	41.8	1.2	2.4	45.0	9.6	26.0	46.5	24.0	3.0	0.5	
91	42.6	2.4	2.0	38.6	14.4	34.5	44.0	18.0	3.0	0.5	
92	31.9	2.2	2.1	49.2	14.6	31.5	50.0	17.0	1.5	—	
93	53.6	1.2	1.0	36.4	7.8	24.5	46.0	24.5	5.0	—	
94	46.4	2.0	1.8	36.4	13.4	25.5	46.0	23.5	5.0	—	
95	35.5	1.5	1.8	53.3	7.9	14.5	47.0	32.0	5.5	1.0	
96	49.6	2.0	3.6	31.0	13.8	15.5	46.5	34.0	3.5	0.5	
97	40.8	2.2	2.2	49.0	5.8	20.0	46.0	28.0	5.5	0.5	
98	59.6	3.6	7.6	25.4	3.8	18.5	47.0	28.0	6.0	0.5	
99	54.0	1.4	2.4	25.2	17.0	23.0	47.5	26.5	3.0	—	
100	38.8	1.7	2.5	30.2	26.8	9.5	49.0	32.0	8.5	1.0	
101	43.8	3.0	4.8	25.8	22.6	14.5	43.5	36.0	5.0	1.0	
102	36.4	2.9	5.1	49.1	6.5	24.5	47.0	25.5	3.0	—	
103	54.6	1.4	1.6	32.8	9.6	23.0	49.5	25.5	2.0	—	
104	54.2	2.8	2.2	28.0	12.8	31.5	53.5	14.0	1.0	—	
Average	51.06	2.90	1.95	32.10	11.97	30.03	44.00	21.23	4.30	0.43	74.01

The average number of lymphocytes (32·1 per cent.) was definitely increased, and the percentages in the individual counts varied between 13·4 and 53·3 per cent. These observations are in agreement with those of previous investigators, namely, that the relative number of lymphocytes in the blood of native races in the tropics is definitely increased.

The relative number of the eosinophile leucocytes was larger than in Europeans; the average was 11·97 per cent. and the range was between 0·4 and 31 per cent.; 57 of the counts were above 10 per cent.

It is impossible to determine how far this eosinophilia can be accounted for by helminthic infections, since examinations of the faeces for the presence of intestinal parasites were impossible; but Bahr has pointed out that eosinophilia was well marked in the blood of Fijians, who showed neither ova of intestinal parasites in the stools nor microfilariae in the blood. Eosinophilia, as is well known, may be due to other causes than infections with helminths, such as skin diseases and the presence of ectoparasites, both fairly common amongst the natives.

The relative numbers of transitional and large mononuclear leucocytes corresponded on the whole to figures considered normal for Europeans.

The differential counts of 50 children (see Table II) showed similar but more pronounced changes. The average number of polymorphonuclear leucocytes was 40·06 per cent., with a variation in the individual counts between 18·4 and 65 per cent.

There were on the average 42·86 per cent. lymphocytes, and no count showed less than 29 per cent.

The average number of eosinophile leucocytes was increased (13·24 per cent.), the individual counts ranging between 1 and 42·4 per cent.

There was no alteration in the relative number of large mononuclear and transitional leucocytes.

TABLE II.

Differential and Arneth Counts on the Blood of 50 Native Children from New Guinea.

Differential Counts per cent.						Arneth Classification per cent.					
Number	Polymorpho- nuclear	Transitional	Large Mononuclear	Lymphocytes	Eosinophiles	I	II	III	IV	V	Arneth Index
1	40.2	0.8	0.8	52.4	5.8	44.0	42.0	13.0	1.0	—	
2	41.0	0.8	1.8	51.4	5.0	31.0	46.0	20.0	3.0	—	
3	30.9	0.9	0.9	61.9	5.4	37.5	40.5	20.0	2.0	—	
4	52.2	2.6	1.4	38.4	5.4	21.5	42.0	26.5	8.0	2.0	
5	37.6	1.1	1.3	47.6	12.4	41.5	43.0	14.0	1.5	—	
6	32.3	1.7	1.4	53.1	11.5	40.0	44.0	14.5	1.5	—	
7	36.4	1.4	0.9	54.6	6.7	39.5	35.0	22.5	3.0	—	
8	26.9	1.5	0.9	59.5	11.2	48.0	40.0	12.0	—	—	
9	40.8	3.8	1.4	30.0	24.0	28.5	32.5	25.0	11.5	2.5	
10	31.0	3.6	2.2	32.6	30.6	32.0	40.0	21.0	6.5	0.5	
11	37.5	2.0	0.5	36.7	23.3	29.0	40.0	23.5	7.0	0.5	
12	28.6	4.1	0.7	34.0	32.6	33.5	43.0	19.0	3.5	1.0	
13	46.6	4.0	6.6	39.0	3.8	44.5	41.5	12.0	2.0	—	
14	50.0	4.3	2.1	36.0	7.6	43.0	44.5	12.0	0.5	—	
15	52.6	4.6	1.6	34.0	7.2	61.0	36.0	3.0	—	—	
16	39.0	1.6	2.0	43.4	14.0	35.5	46.5	14.5	3.5	—	
17	46.0	2.8	1.6	38.2	11.4	38.0	37.0	19.0	5.5	0.5	
18	33.3	1.5	0.2	49.2	15.8	49.0	39.5	9.0	2.5	—	
19	27.9	2.4	1.5	50.6	17.6	34.5	48.0	16.5	1.0	—	
20	38.3	3.7	1.0	41.3	15.7	35.0	46.5	15.5	2.5	0.5	
21	51.4	2.6	0.6	35.2	10.2	61.5	31.5	7.0	—	—	
22	28.6	2.0	2.5	39.2	27.7	44.5	43.5	11.5	0.5	—	
23	22.7	2.0	0.3	53.3	21.7	32.0	48.0	18.5	1.5	—	
24	47.6	1.0	2.0	42.8	6.6	53.0	39.5	6.5	1.0	—	
25	41.2	2.2	2.8	48.6	5.2	44.0	48.5	7.5	—	—	

TABLE II.—Continued.

Number	Differential Counts per cent.					Arneth Classification per cent.					Arneth Index
	Polymorpho- nuclear	Transitional	Large Mononuclear	Lymphocytes	Eosinophiles	I	II	III	IV	V	
26	40.6	1.4	1.8	44.6	11.6	47.5	42.5	9.0	1.0	—	
27	36.8	1.5	1.5	42.4	17.8	39.5	46.5	13.0	1.0	—	
28	35.3	1.3	2.2	54.7	6.5	42.0	46.5	11.0	0.5	—	
29	53.4	2.2	1.4	32.6	10.4	29.5	48.0	20.0	2.5	—	
30	37.3	1.1	1.4	47.1	13.1	48.0	40.0	12.0	—	—	
31	41.8	1.4	1.6	45.8	9.4	24.5	44.5	25.5	5.5	—	
32	32.7	1.3	0.8	54.2	11.0	30.5	46.5	21.5	1.5	—	
33	28.9	0.9	1.0	56.1	13.1	39.5	44.0	14.5	2.0	—	
34	40.2	2.2	0.8	31.2	25.6	46.0	40.5	12.0	1.5	—	
35	25.0	4.8	3.1	55.4	11.7	57.0	34.0	8.0	1.0	—	
36	35.3	2.0	1.4	39.5	21.8	55.5	37.0	6.0	1.5	—	
37	45.4	1.6	1.4	40.2	11.4	51.0	33.5	14.5	0.5	0.5	
38	43.0	3.2	—	38.2	15.6	52.5	36.0	10.5	1.0	—	
39	41.2	4.0	1.2	42.0	11.6	36.0	45.0	16.5	2.5	—	
40	54.8	3.8	—	37.4	4.0	45.0	45.0	8.5	1.5	—	
41	18.4	1.3	—	37.9	42.4	49.0	40.0	8.0	3.0	—	
42	38.0	1.3	0.5	48.4	11.8	42.0	42.5	12.5	2.5	0.5	
43	45.3	8.8	1.0	38.7	6.2	41.5	37.5	17.5	3.0	0.5	
44	37.1	3.0	1.5	38.8	19.6	60.0	35.0	4.0	1.0	—	
45	40.4	2.6	—	38.2	18.8	42.5	44.0	13.0	0.5	—	
46	59.0	3.8	1.8	30.6	4.8	44.0	42.0	12.0	2.0	—	
47	65.0	4.2	0.8	29.0	1.0	58.0	35.0	7.0	—	—	
48	53.2	3.0	1.4	35.4	7.0	56.5	36.5	7.0	—	—	
49	45.4	2.4	1.2	49.4	1.6	60.5	29.5	9.5	—	0.5	
50	49.0	1.6	1.2	32.4	15.8	48.5	35.0	13.5	2.0	1.0	
Average	40.06	2.47	1.36	42.86	13.24	42.96	40.90	13.80	2.13	0.21	83.86

### ARNETH COUNTS

For the Arneth count 200 consecutive white cells were enumerated in two separate lots of 100, and only those counts were considered where there was close agreement between the two series.

The average for the Arneth index (the sum of leucocytes of classes I and II) in 104 adult natives was 74 (see Table I). A comparison with the average index of Europeans (40) shows that the index is considerably increased in adult New Guinea natives. It is practically the same as that in North Queensland school children (74.5), and is higher than that found by Chamberlain and Vedder in their examination of 50 Filipinos (65.8).

The average Arneth index of 50 New Guinea children (1 to 10 years old) was still higher, being 83.86 per cent. (see Table II).

This close correspondence between the Arneth index of adult New Guinea natives and that of North Queensland school children indicates that the increase in natives cannot be attributed to infection only, since the school children examined were, as far as one could judge, perfectly healthy.

The comparative increase in the Arneth index of native children, when compared with adults, probably finds its explanation in the greater incidence of active and latent infection amongst native children. Yaws and malaria were found endemic in the majority of the coastal villages of British New Guinea, and although children who were obviously suffering from yaws, and those who had malarial parasites were excluded, it is probable that the majority of the children were, or had been, suffering from either malaria or yaws, or most likely both.

This assumption is, moreover, strengthened by the observations of Scott Macfie on the Arneth counts of cases of malaria in West Africa. The blood of such cases showed an increased Arneth index, which persisted even after the disappearance of the parasites from the blood and the recovery of the patient.

### CONCLUSIONS

1. Differential counts on 104 adult natives of New Guinea show a decrease in the number of neutrophile leucocytes and an increase in the number of lymphocytes and eosinophiles.



2. Differential counts of 50 native children show similar but more pronounced changes.
3. Arneth counts of adult natives show a marked shift to the left, the average Arneth index being 74.0.
4. Arneth counts of 50 children gave an average Arneth index of 83.86.
5. The identity of the Arneth index of adult natives with that of healthy school children in North Queensland strengthens the assumption that climatic influences, *per se*, cause a 'shift to the left'; but the still greater shift in native children is probably due to active or latent infections.

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# A CASE OF DYSENTERY IN A MONKEY, IN WHICH AMOEBAE AND SPIRO- CHAETES WERE FOUND

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## PLATE XXXVII

### INTRODUCTION

A small monkey, *Cercopithecus petaurista*, in which a strain of the human trypanosome had been sent to Accra, Gold Coast Colony, from Sunyani in June, died unexpectedly on August 5th, the sixty-fifth day after inoculation. The monkey had been observed to be suffering from diarrhoea for some time, but as it appeared to be healthy otherwise, little attention was paid to this fact.

At the autopsy the body was found to be somewhat wasted, and all the organs and tissues were decidedly anaemic. The lungs appeared to be healthy. The heart was pale and flabby, and the blood was unusually thin. In the abdominal cavity there was no excess of peritoneal fluid; the spleen was considerably enlarged but the other organs were healthy looking, but anaemic. There was no hypertrophy of the lymph glands. No parasites were found in the gut, but throughout its length the large intestine was congested and studded with ulcers. These ulcers were most numerous near the caecum, but were scattered all over the mucous surface down to the rectum. Each ulcer was roughly circular, with a central slough, and raised, ragged, blood-stained edges (see Plate XXXVII, fig. 12).

In the blood trypanosomes were found, but they were not numerous. During the forty days the monkey had been under observation at Accra the trypanosomes had always been scanty or rare in the blood, and no symptoms of trypanosomiasis had manifested themselves. It is improbable that the death of the

monkey was due to trypanosomiasis. The short duration, sixty-five days since inoculation, the absence of all clinical symptoms, and of enlargement of the lymph glands, and the fact that another small monkey inoculated at the same time is still alive and well although more heavily infected, all tend to prove that death must have been due to some intercurrent disease. The extensive ulceration of the large intestine suggests itself as this cause, and I believe that death was due to amoebic dysentery.

#### AMOEBAE FOUND IN THE LARGE INTESTINE

In fresh preparations of the contents of the large intestine numerous amoebae were seen. They were very active, extruding clear pseudopodia in all directions from their surfaces, but did not progress rapidly across the field of the microscope. In each a single nucleus was visible through the granular and vacuolated endoplasm, and in some cases red blood corpuscles were seen to have been ingested. There was no contractile vacuole. When stained the amoebae measured from  $12\mu$  to  $30\mu$  in diameter (Plate XXXVII, figs. 1-6). The cytoplasm was much vacuolated and contained numerous bacteria-like chromidia. The nucleus was somewhat indistinctly stained by Leishman's method, but appeared to possess a fine chromatin network, but no distinct karyosome. In considering this fact it should be remembered that the specimens were obtained at an acute stage of the infection. The commonest forms of the amoebae measured  $12\mu$  to  $15\mu$  in diameter; but a few much larger individuals were found which measured  $26\mu$  to  $30\mu$  (fig. 3). The latter forms, besides being vacuolated, often contained a number of ingested red corpuscles, and in some cases this may have accounted for their large size. It is possible, however, that the large amoebae might have been of a different species; but as some forms of intermediate size were also present, it seems more probable that all the forms were stages in the life cycle of the same parasite. Numerous cysts (figs. 7-10) were also present measuring as a rule  $12\mu$  to  $18\mu$  in diameter, but some larger forms were seen which measured as much as  $33\mu$ . These cysts had a thick wall, which stained red with Leishman's stain, and a single large (? glycogen) vacuole occupying the greater part of the interior. The

vacuole stained a pink colour. In the narrow band of cytoplasm the nuclei were visible, as oval or rounded bodies with a palely stained chromatin network. The number of nuclei varied greatly; in some cysts there was only one, but as a rule there were several. In one cyst eight nuclei were counted (fig. 10). In sections large numbers of amoebae were seen in the bases of the ulcers and penetrating into the adjacent layers of the intestine.

The cysts were in some respects unlike amoebic cysts, and resembled superficially the bodies called 'cysts of *Trichomonas intestinalis*' which Alexeieff (1911) considers to be 'en réalité un Ascomycète voisin des Levures,' and for which he has proposed the name *Blastocystis enterocola*. If Alexeieff is correct in his interpretation, it is possible that the cysts found in this monkey at Accra may have been of a similar nature. It should be mentioned, however, that the characteristic nuclear structure described by Alexeieff, 'une calotte chromatique périphérique séparée par un halo clair du reste de la substance chromatique finement granuleuse,' was not observed; and no indications were seen of the two forms of division traced by him, namely, that by budding ('bourgeonnement'), and that 'par étranglement (division plasmotomique).' Neither did the cysts appear to be enclosed in a mucilaginous covering as *Blastocystis enterocola* apparently is. It seems more probable therefore that, in the case of this monkey, the cysts should be associated with the amoebae that were so numerous in its intestine.

Amoebae have previously been described from various monkeys, Mathis (1913) described two types of cysts found in healthy monkeys (*Macacus rhesus* and *Macacus tcheliensis*) in Tonkin, and gave a résumé of the literature on this subject and enumerated the Entamoebae found by various authors in monkeys. None of these parasites seems to have resembled those found in this case. Swellengrebel (1914) described by the name *Entamoeba chattoni* an amoeba found in *Macacus rhesus* at Deli. This parasite measured in the amoeboid form between  $13\mu$  by  $12\mu$  and  $12\mu$  by  $9\mu$ , and in the cystic  $9\mu$  or  $8\mu$  in diameter. The cysts were described as containing a glycogen vacuole, but only uninucleate and binucleate forms were observed. A comparison between the illustrations given by Swellengrebel, and those on the plate attached

to this note, will show, I think, that the amoeba from *Macacus rhesus* differed from that found in *Cercopithecus petaurista*. In the majority of cases the parasites have been found in healthy animals, and their pathogenic nature is doubtful. I have not been able to find any previous description of a case of amoebic dysentery in a monkey (*Cercopithecus*) similar to the above, and I would therefore suggest the name *Entamoeba cercopitheci* for the parasite found in this case.

**A SPIROCHAETE RESEMBLING *SPIROCHAETA EURYGYRATA*  
FOUND IN THE LARGE INTESTINE OF THE SAME MONKEY**

In addition to the amoebae, vast numbers of minute spirochaetes were seen in the smears made of the contents of the large intestine and rectum of this monkey (fig. 11). These spirochaetes were extremely slender, and had finely-pointed extremities. They stained uniformly a reddish colour with Leishman's stain. When living they were very active, but owing to their small size were difficult to study. Twenty-five individuals, taken as they came, were drawn with a camera lucida and measured by the tangent line method. The average length was  $5.28\mu$ , and the range from  $4\mu$  to  $7\mu$ . Some of the spirochaetes consisted of a single loop or spiral, others showed two and a half such coils, but the majority showed one and a half or two, and measured  $5\mu$  to  $6\mu$  in length (see Table 1).

TABLE I.—Measurements of twenty-five specimens of a spirochaete found in the large intestine of a monkey.

Length in microns	Number measured	Average number of loops or spirals
4	5	1.3
5	10	1.6
6	8	1.87
7	2	2.25

The morphology of these spirochaetes closely resembled that of *Spirochaeta eurygyrata*, a species found by Werner (1909) in his own faeces at a time when he was apparently in good health. Similar

spirochaetes have been found by J. G. and D. Thomson (1914) in the stools of apparently healthy individuals; and, at Accra, I have found what would appear to be the same parasites in enormous numbers in the faeces of two patients who were suffering from diarrhoeic symptoms. *Spirochaeta eurygyrata* is said to measure  $4.6\mu$  to  $7.3\mu$  in length, with usually two curves, and to possess a very flexible body; a description that would apply very well to this spirochaete from *Cercopithecus petaurista*, and it would, no doubt, be inadvisable to give the latter parasite a specific name merely because it occurred in a different host.

There was no evidence in this case that the spirochaetes were pathogenic, and it is probable that they were comparable with the small spirochaetes frequently found in the gut of animals, and man, in West Africa. It is not improbable, however, that the debilitated condition of the host, and the diseased state of the large intestine, may have provided the favourable medium which allowed these organisms to multiply; and that the vast number of spirochaetes thus engendered may have contributed to the ill-health of the animal which finally culminated in its death.

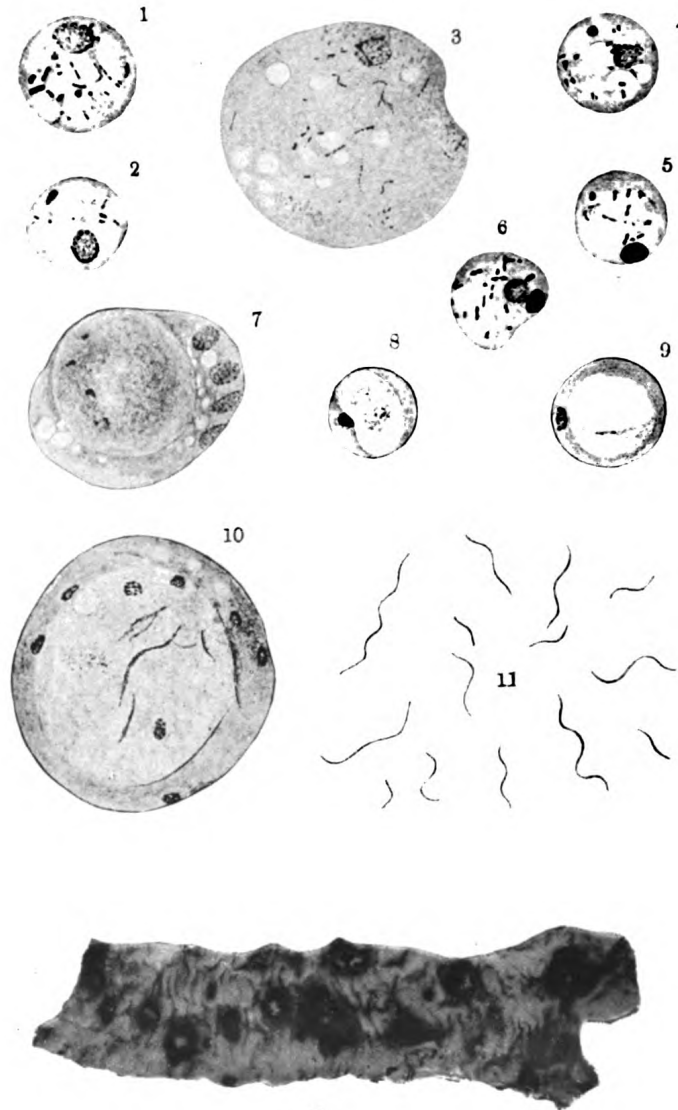
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## EXPLANATION OF PLATE XXXVII

- Figs. 1-10. *Entamoeba cercopithecii*, n.sp., from a monkey (*Cercopithecus petaurista*) at Accra, Gold Coast, West Africa. Figs. 1-6, vegetative forms; figs. 7-10, cysts.  $\times 1000$ .
- Fig. 11. A spirochaete of the type of *Spirochaeta eurygyrata* from the large intestine of the same monkey.  $\times 2000$ .
- Fig. 12. A piece of the large intestine of the monkey showing the ulcers,  $\frac{3}{4}$  natural size.





*M. Rhodes, del.*

AMOBÆ AND SPIROCHAETES,  
FROM THE GUT OF A MONKEY.



PRELIMINARY NOTE ON THE  
GENERAL DISTRIBUTION OF *GLOSSINA*  
*PALPALIS*, ROB-DESV., IN THE DISTRICT  
OF LOMAMI, BELGIAN CONGO

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(Received for publication 12 May, 1915)

WITH MAP

Three principal factors determine the distribution of tsetse-flies in any region; first the climate; secondly the vegetation, whether forest, 'park,' 'bush,' savannah, or steppes; and thirdly, the distribution of water, such as lakes, rivers, streams and marshes. Upon the variable combination of these factors depend not only the presence or absence of all tsetses, but also of particular species or groups. Knowing then the three factors in question for a Central African region, it is possible to foresee and to predict the presence or absence of one or other group of tsetses.

The district of Lomami occupies the region situated between 5° and 9° S. latitude and between 23° and 26° E. longitude. Thus it is a tropical country with two seasons more or less marked according to the latitude. The altitude varies from 1,000 metres in the South to about 500 metres in the North. If from an administrative point of view Lomami belongs to Katanga, it is separated both geologically and botanically therefrom. Here it would be out of place to discuss the geological differences, notably the absence of mines, but I would recall that, as regards vegetation, Katanga is characterised by an almost continuous 'park' (or bush as it is termed in Katanga) which is interrupted only by grassy plains (savannah or steppes) on the higher plateaux. Only the S.E. border of the Lomami district partakes of the park-like nature of Katanga, the remainder being occupied by more or less typical savannah

intersected here and there by varying sized belts, along the rivers, and by stretches, in the lower parts, of true equatorial forest. However, in certain regions (for example between Mutombo-Mukulu and Kabongo) areas recalling the 'park' are found, but these are isolated and do not form large continuous stretches as in Katanga proper.

The terminology used in connection with these different types of vegetation is very embarrassing and often leads to confusion. Each author interprets the various terms in his own fashion, and in certain publications the explanations of 'bush' (*brousse*), 'wooded savannah' (*savane boisée*) are so vague that it is impossible to understand them. This is chiefly due to the fact that nature is infinite in its variations and lends itself by no means easily to our schematic definitions. Such schemes are, however, useful in facilitating the comprehension of the phenomena and thus in systematising our ideas. Therefore, I am constrained to give my own interpretation of the different names applied to the various forms of tropico-equatorial vegetation. First, I would mention that the word 'bush' does not define any particular type of country; in African usage it indicates any area outside the station, and is therefore a general term with a somewhat negative significance.

There is little need to give any definition of the term *equatorial forest*. Everyone knows that it is a collection of very tall trees whose upper branches give continuous and permanent shade and whose trunks are covered with lianas and creepers and surrounded with bushes and small trees, forming an inextricable and impassable barrier. In the regions of the savannah, at least in Lomami, the forest is encountered in the lower swampy parts and along the rivers, forming the so-called *galeries boisées* along the latter. By the term 'park' ('*parc*') is meant an area covered with trees, usually stunted and deformed, which provides but little shade; lianas and creepers are almost entirely absent, and travelling between the trees is easily accomplished. The *savannah* (*savane*) is a flat or undulating stretch of country covered with grass; if, however, the grass is short the term *steppe* is usually applied. The savannah is rarely covered with grass alone; as a rule, groups of bushes, small trees and shrubs occur, and sometimes these bushes, etc., are numerous and fairly tall, in which case the term *wooded savannah* (*savane boisée*) or

*bush (brousse)* is used. The wooded savannah thus represents a type intermediate between the true savannah and the park.

It will be remembered that all tsetse-flies require shade. While *G. morsitans* has need of a certain freshness and does not require the immediate proximity of water, *G. palpalis* must have a warm and damp atmosphere, and the immediate presence of water in some form—lakes, rivers, swamps, or, at least, very small marshes. *G. morsitans*, in fact, *exclusively inhabits the 'park,'* while *G. palpalis* frequents exclusively the immediate neighbourhood of water surrounded—or even covered, as in marshland—with dense vegetation. Therefore, in all stretches of true equatorial forest *G. palpalis* may be everywhere, while in the savannah regions it is able to exist in the galleries boisées along the water courses, and in the lower wooded parts. *The savannah itself is always exempt from tsetse-flies.* *G. morsitans* is to be found neither on the plains nor on the plateaux, and *G. palpalis* does not occur in the neighbourhood of water when the latter is bordered only with grass, reeds or papyrus.

Following the above explanatory remarks it will be sufficient to add for the preliminary comprehension of the general distribution of *G. palpalis* in the Lomami district, that the whole region is intersected with thousands of wooded watercourses and is covered with numerous lakes, lagoons and marshes of varying size, all of which are also often wooded. In certain regions of Africa, notably Katanga, *G. morsitans* is, among other things, characterised by its ubiquity, while in Lomami *G. palpalis* occurs only locally.

Almost all my ideas regarding the habitats of this tsetse-fly—ideas which I had acquired during my first stay in the Congo, notably at Tanganyika—have been upset after several months' travelling in Lomami. Certainly the general and well-known principle, that the existence of *G. palpalis* depends upon two fundamental conditions, viz., the presence of water and shade-giving vegetation, remains, and will remain, unaffected, but a principle of so general a nature is only sufficient for amateurs. Formerly I had elaborated certain fundamental principles which enabled me to predict, on approaching a place, whether *G. palpalis* would be present or not—and I was seldom mistaken. Here, however, the principles upon which I relied seemed to collapse entirely. For

example, I held previously that all small rivers or streams were exempt from *G. palpalis*, and now I found it often occurred on quite insignificant watercourses. Further I had stated that in very marshy places there were no *G. palpalis* (and this fact has been verified by numerous observers in East Africa), but here I discovered it in great numbers in the deep marshes and on the swampy papyrus-covered borders of rivers. Finally, to my great amazement, I was also attacked by *G. palpalis* among certain very small and insignificant swamps. But should we infer that the habitat of this tsetse-fly is totally different in different regions? By no means—it is merely that certain adaptations to local conditions occur which require analysis and interpretation.

It may be of interest if I discuss a few of the peculiar cases which have thus disconcerted me.

#### I. *Lake Boya* (or *Moria*)

About two hours' journey from Kabongo is a small lake, more or less ovoid in form and approximately 4 to 6 kilometres in diameter. This lake is situated in a swampy hollow, and the approach to it is rendered very difficult, and in many places absolutely impossible. It seems that until quite recently (about three years ago) there existed a true lacustral village built on piles, but now there remain only three small fishing villages situated near the lake. Leaving one of these villages in order to make a short tour of the lake in a native boat, I walked in the forest for a time and suddenly arrived at an abrupt bank, sharply dividing the forest from a marsh covered with papyrus and other plants. I descended to the marsh, a difference in level of several metres, and preceded by some natives who pointed out the safe spots on which to tread, commenced to flounder in an evil-smelling slime. The marsh presented the appearance of a series of small islands of papyrus and other plants, disseminated on the surface of a gelatinous substance of a semi-solid or semi-liquid nature. The surface of this mass was for the most part bound together by the superficial roots of the vegetation, where this happened to exist, but the further one progressed the more difficult and perilous became the advance, and one was in danger every moment of being engulfed. Ultimately a narrow channel, made by the natives, was reached, and a pirogue or native boat obtained. This

canal enables the natives to traverse the intermediate zone between the lake proper and the surrounding marsh. In reality there is no distinct limit between the lake itself and this intermediate semi-liquid zone; the latter gradually becomes more and more liquid the nearer one approaches the lake. A glance at the surroundings was almost sufficient to convince me that Lake Boya was exempt from *Glossina palpalis*. But while standing on the edge of the channel awaiting the return of the natives, who had gone to look for a second boat kept in a neighbouring channel, I perceived to my great astonishment that a specimen of *G. palpalis* had settled on myself—shortly afterwards also I noticed a second example on a native. During my tour of some hours on the lake, in which both the centre and the banks were visited, no further specimens were seen in spite of most careful search; neither were any visible during my subsequent return on foot across the marsh. When, however, I approached the steep bank at the edge of the forest belt, I suddenly perceived several tsetses, and later found that they were very numerous in the forest itself. The explanation of the appearance on the marsh of the two flies was thus provided. On the immediate borders of the lake, that is, in the marsh covered only with plants—papyrus and occasional reeds—there are no flies whatever. This marsh, however, replaces the reservoir of water required, and when, as in the proximity of the forest belt, shade-giving vegetation is present, the conditions necessary for the existence of this species are supplied. The apparent absence of the fly from the forest region during my first incursion on approaching the lake was undoubtedly due to the earliness of the hour. The two flies encountered in the marsh therefore had evidently followed me and my black companions from the forest.

## 2. *River Kekey*

This river, a left affluent of the River Lomami, presents during the greater part of its course special characters recalling those of Lake Boya. In the neighbourhood of the villages Mulenda and Inga (Kabinda-Kisengwa route) the river itself is not visible; a long valley about one kilometre wide with distinct, more or less elevated banks can be seen. This valley is in reality a deep marsh covered with grass, reeds and papyrus, and bordered on each side at

intervals with trees of various sizes, bushes, etc. Within this valley, sometimes in the centre, sometimes near one or other side, winds the Kekey river—a river of some little importance. Near the village Mulenda, where the 'marsh-valley' is very deep and dangerous, is an artificial canal commencing at the border of the valley itself, and used by the natives for fishing purposes. As it happens, the marsh is very rich in a species of eel, which constitutes an important branch of the local commerce. The river also supplies a species of trout.

*G. palpalis* is more or less abundant on the edges of this marsh. But another question presents itself: is the fly to be found on the banks of the river itself, that is in the centre of the marsh? This is without cover, there are no trees or bushes, and theoretically the fly ought not to be present, but as a native boat was unobtainable in order to reach the river viâ the canal, I was forced to forego personal observation. Judging by what I have seen since under absolutely identical conditions on the River Lomami, I conclude that *G. palpalis* is not in evidence on the River Kekey itself.

### 3. *River Lomami*

This river is of great length, and traverses successively, wooded, grassy, forestal, mountainous, swampy and open flat regions; accordingly it presents much variation in the formation of its banks. In the region of the 5th parallel the Lomami runs between two walls of forest, while in the neighbourhood of the 7th parallel the banks of the Lomami are in the form of papyrus-covered swamps recalling those of the River Kekey. At a certain distance from the river a chain of more or less wooded hills is visible on each side. In places, however, these hills approach quite close to the river, receding again later to give place to the marsh.

No single specimen of *G. palpalis* was seen so long as the river flowed between the swampy banks, but directly the course approached woody and hilly borders, the fly appeared and continued in evidence until the marshy, papyrus region was re-entered.

In all the above places then—Lake Boya, the River Kekey and the River Lomami—the same thing occurred. The marshy, uncovered banks, whether of lake or river, are exempt from *G. palpalis*, but such marshes are the equivalent of open water, and, therefore, when bordered with shade-giving vegetation, the conditions necessary for the existence of the fly are present.



About one hour from Thielen St. Jacques (in the Kanda-Kanda territory) is a large village Tshipama. Five minutes' walk from this village is a kind of spring from which the natives draw water; it consists of two or three holes in excessively damp ground from which water trickles and runs in insignificant streams to the neighbouring heavily-wooded ground, which is thus rendered so swampy that on entering, one is immediately immersed up to the knees. There is thus no expanse of open water, but further on, owing to the slope of the ground, the small streamlets unite to form a brook—a secondary or tertiary tributary of the large river Luilu, which itself is a tributary of the Lubilash-Sankuru.

I was much surprised to find numerous examples of *G. palpalis* in this wooded swamp near the spring, as it was the first time that I had met the fly under such conditions, namely, in a small wooded swamp *without the immediate presence of an uncovered mass of water*. Since then, however, I have often found *G. palpalis* even in the smallest swamps, where the necessary vegetation existed. Therefore, so long as a place possesses shade and a certain degree of humidity, *G. palpalis* can exist, although it is not necessarily present. Even permanent humidity is not essential, since temporary humidity, as in the rainy season, will suffice. As soon as this humidity disappears in the dry season, the fly disappears also, only to reappear later when conditions are favourable. This reappearance may be brought about in two distinct ways, firstly when conditions become unfavourable in any region, *G. palpalis* is able to migrate to a more favourable locality where there is permanent water, and later to return to its former habitat with the change of the season; secondly the conditions having become unfavourable to its existence the fly dies, but there remain in the ground numerous pupae (for the development of which dryness is required) which furnish adults for the subsequent more suitable period. These two phenomena take place, according to circumstances, separately or even simultaneously, for the preservation of the species.

It is only necessary to recall what has been stated above, viz., that the district is bestrewn with marshes and swamps of various sizes, that it is intersected with numerous watercourses, and that the lower parts, where all these occur, are generally wooded, for us to understand that the semi-ubiquity of *G. palpalis* in this region is very natural. Happily, however, there are many exceptions to this,

although one may not always be able to explain the reasons for them. In certain regions, for example in the territories of Kanda-Kanda and Tshofa, *G. palpalis* occurs on the majority of the streams, while in the Kasongo-Niembo region the fly is relatively rare. From an examination of several rivers on numerous occasions and in different places, I have been able to deduce the following rule:—*If a known spot on a river is positive as regards the presence of G. palpalis, then any others situated down-stream, on the same river, are also positive (provided always there is shade-giving vegetation); the character of places situated up-stream is unknown, and may be positive or negative.* Further, if any place is negative, points higher up the river are also negative, but points lower down are unknown.

#### DIFFICULTIES ENCOUNTERED IN EXAMINING A REGION FOR *GLOSSINA PALPALIS*

The information here given on this subject may perhaps be of some value to other medical men, and to those in general who are interested in this question; it may even help them to avoid certain errors.

On the 4th May, 1913, when travelling from Mwana-Tonto to Tutu (via the Kanda-Kanda to Mutombo-Mukulu route) it was necessary to cross the large river Luilu, which I reached at 7 o'clock in the morning, one hour after sunrise. Rain had fallen on the previous evening, and it was damp and fresh; no insects were visible. About 7.30 the animal world commenced to awake; first came the 'ordinary' flies, then some butterflies, afterwards various hymenoptera (small bees, wasps, carpenter bees), and horse flies (*Tabanus*), but it was only at 9 o'clock that the first specimen of *G. palpalis* appeared and not until 10 o'clock did they become numerous. I would, therefore, not have seen a tsetse-fly if I had left the river before 9 a.m., even had I remained there for a considerable period, and consequently one might easily have stated that *G. palpalis* was not present in this spot.

The same thing occurred on the River Lomami along the Mutombo-Mukulu to Kasongo-Niembo route, where I arrived at 8 o'clock in the morning on the 24th May. It was the beginning of the dry season, and was cold in the night and early morning; at this

hour (8 a.m.) the dew had not yet disappeared, it was very fresh beneath the trees on the river banks, and all was quiet. I waited—first appeared some ants on the tree trunks and some of the large Tipulids, then successively 'common' flies, small moths, butterflies, carpenter bees and, at last, at 9 o'clock the first *G. palpalis*. It was not until nearly 9.15 a.m. that the second appeared, but at 10 o'clock these flies were numerous and commenced to bite.

I reached the River Lovoi (when travelling on the Kikondja-Kabongo route) late in the evening of the 4th February, and installed myself near the bank. It rained continually throughout the night, and although, from an early hour in the morning, I repeatedly visited the river bank, I had not seen a solitary tsetse-fly by 11 o'clock. Naturally under the circumstances, I should have concluded that no *G. palpalis* were present here at all, but for the fact that I remained in this place for the rest of the day and then saw the flies appear in the afternoon. After heavy and prolonged rain, then, during which the temperature is lowered and everything saturated, *G. palpalis* remains hidden and inactive for a considerable period.

The River Lukula is a tributary of the River Lubefu. It is narrow and deep (about 5 metres in width) with a very rapid current, and it flows between high and steep banks covered with dense vegetation. The upper branches of the trees on each side are in contact, and form a complete roof over the stream. I crossed the Lukula, in its upper regions between Mwana-Kialo and Bukile, on the 10th July, but was not able to discover a single *G. palpalis* in spite of the fact that I remained in this spot for one hour at a very suitable time of the day (11 o'clock to noon). On the 22nd July, I re-crossed the Lukula between Dibwe and Lubefu, that is to say, not very far from the mouth. Every July a dense mist occurs in the mornings, which only disperses later in the day, about 8 or 9 or even 10 o'clock. It was on such a morning that I reached the river. In spite of the relatively late hour, 9 o'clock, the mist had not yet cleared on the well-wooded banks of the river, and it was still cool under the canopy formed by the upper branches of the trees. I remained an hour on the river, but saw no traces of the fly. In spite of this, and of the fact that I had not found *G. palpalis* in the upper regions of the river under exactly similar

conditions, I was not satisfied. This spot was so near the mouth that it would certainly be strange if *G. palpalis* were not present. It was already between 9 and 10 o'clock, and elsewhere was quite warm, but on the river it was still fresh since the sun's rays had not yet thoroughly penetrated the overhanging branches. Although it is generally stated that this fly is active from sunrise, I waited, and was well rewarded for doing so, as about 11 o'clock I heard the typical and peculiar humming of the first tsetse-fly. I may mention, in this connection, that when travelling on the River Lomami in a native boat, between Tshofa and Gandu, from the 9th to 11th August, *G. palpalis* appeared about 7.30 a.m., in spite of the morning mist. The Lomami, however, at this spot is more than 100 metres wide, and, consequently, its surface is quickly warmed by the sun, and the fly appears earlier than on the Lukula.

At 7 o'clock on the morning of the 21st May, I crossed the large river Luembe on the Mutombo-Mukulu to Kabongo route. No specimens of *G. palpalis* were to be seen, and, unfortunately, I was unable to remain for any length of time. Would it be more correct to say in consequence that *G. palpalis* did not occur here, or that it was not visible owing to the earliness of the hour? The answer is very simple. The previous evening, I stopped in a village situated about half an hour's walk from the river, and, foreseeing what might happen, went in the afternoon to examine the river, and there saw numerous specimens of *G. palpalis*.

Between the station Kanda-Kanda and the mission of Thielen St. Jacques are three small streams, on all of which *G. palpalis* occur. Between Thielen and the hamlet Pokote are also three small streams and a little brook, and on all of these again the fly is found. My two visits to these spots were paid between 10 a.m. and 1 p.m., and 10 a.m. and noon, respectively. When some days later, in May, I crossed two small rivers, surrounded by forest vegetation, between Mwana-Msenge and Mwana-Tonto no trace of *G. palpalis* was to be found. The only difference was that I had halted here early, and had crossed the streams between 7 and 8 a.m.

I could continue to give similar examples, but consider it unnecessary, as the few facts cited prove sufficiently that much care is required. It is evident that, if one remains for a long time in one spot, and examines it on numerous occasions with the result that,

under all conditions, morning, noon and evening, in the dry season and in the rainy season, no sign of the tsetse-fly occurs, then it can be stated that the fly is absent. But without such precautions one must not be too dogmatic. From a casual examination it is only possible to say that *G. palpalis* has not been seen, but this does not necessarily mean that it is not present. In fact the statement, that this tsetse-fly is active from sunrise to sunset, is incorrect; *G. palpalis* flies and bites during the day when the temperature is not too low and when the surrounding vegetation is not too heavily saturated by rain or dew. Other conditions being equal, *G. palpalis* appears earlier and disappears later in the rainy season than in the dry season, as the nights of the latter are relatively cold and the evenings and mornings fresh. A little fine warm rain scarcely affects *G. palpalis* at all, but during, and for a long time after, heavy rain it is not in evidence. A severe night rain consequently retards the appearance of the fly, and heavy rain later in the day, or towards noon, causes it to disappear for the remainder of the day. It is thus necessary to choose for the examination a suitable spot and moment. During the rainy season there are mornings, afternoons, and even entire days which are not favourable. Early morning and evening are never suitable, especially in the dry season, and yet, in the Congo, it is impossible to travel systematically at a period of the day other than the early morning. In general, the presence of *G. palpalis* is discovered quickly enough given a favourable time, for example, mid-day, and often it is not even necessary to search for the flies, as they soon make their presence known—especially when they occur in large numbers. There are cases, however, when one can only discover the tsetse-fly after searching for an hour or more, as the following examples show.

The River Lukashi is an important tributary on the left bank of the River Lomami (another tributary on the right bears the same name), and I have seen *G. palpalis* in March, at the crossing of this river on the Kisengwa-Kabinda route, below the mouth of the River Loamba. In June, on going from Kabongo to Kabinda, I returned to this river at a point higher up stream near the village of Gongo. Here the river is 6 to 10 metres in width instead of being 50 to 60 metres wide as on the route Kisengwa-Kabinda. Near Gongo, moreover, it has high, steep wooded banks. I reached

the river at 11 a.m. when the sun was very powerful and the heat great, and waited in vain for an hour among the trees on the bank for the appearance of *G. palpalis*; it was not until a further half-hour had passed that I heard the characteristic humming noise produced by these flies when on the wing.

The River Lurimbi enters the Lomami at Tshofa and rises near Kabinda, at the foot of the hill where the village of the well-known chief Lupungu is situated. The route Kabinda-Tshofa follows, more or less, the course of Lurimbi. When going from Tshofa to Kabinda opportunities occurred of visiting several places on this river, and on examination *G. palpalis* was found to be present everywhere. I then decided to seek the fly in Kabinda itself, and it was only after waiting a whole afternoon that I succeeded in capturing two examples at the crossing of the river situated about twenty minutes from the station.

It is, therefore, impossible to draw up an exact and detailed map of the distribution of *G. palpalis* in this district, even along those routes taken by me, in spite of the care, attention and interest that I have taken in this question, for the fact of its not being found does not, as we have seen, imply the absence of *G. palpalis*.

From the last two examples cited it is evident that much trouble was involved in discovering *G. palpalis* on the passages of the Lukashi and the Lurimbi. Both these routes—Kabongo-Kabinda and Kabinda-Pania—are much traversed, and, in these cases therefore, the view of Roubaud and other experts that *G. palpalis* is especially abundant at river-crossings much frequented by animals and men, apparently does not hold. I do not contest the accuracy of the cases described by Roubaud and others, yet, although I have seen *G. palpalis* at the river crossings on numerous occasions, I have never observed that it was more abundant in such places than in those which the natives seldom visit. It seems to me, therefore, that strictly speaking these specialised haunts are far from being demonstrated.

The question as to where *G. palpalis* rests during the night, the colder mornings and wet weather is of interest. These hiding places are evidently among the plants or under the leaves of trees, but the exact positions I have been unable to discover. On several

occasions I have examined the under sides of the leaves of various plants, but always without success.

All observers have verified, and many have given explanations of, the fact that among series of captured specimens of *G. palpalis* the males are far more numerous than the females. I have examined, from this point of view, several of my collections of these flies from various localities with the following results:—

No.	Date	Locality	Males	Females	Total
1	July	R. Sankuru (Pania) ... ..	51	42	93
2	August	R. Lurimbi (Tshofa) ... ..	74	54	128
3	„	R. Lomami „ ... ..	243	205	448
4	„	R. Buluy (Piani Tshungu) ... ..	34	22	56
5	„	In pirogue on the R. Lomami (Tshofa-Gandu) ... ..	83	42	125
6	May	R. Lubishi ... ..	36	66	102
7	„	R. Luembe ... ..	14	1	15
8	March	R. Lukashi ... ..	31	11	42
9	„	R. Kela (Inga) route Kabinda-Kisengwa	46	17	63
10	July	R. Lukula ... ..	15	25	40

My own results thus, on the whole, support these statements, but in two cases, Nos. 6 and 10, the opposite occurs, viz., the females, many of which were gravid, are almost twice as numerous as the males.

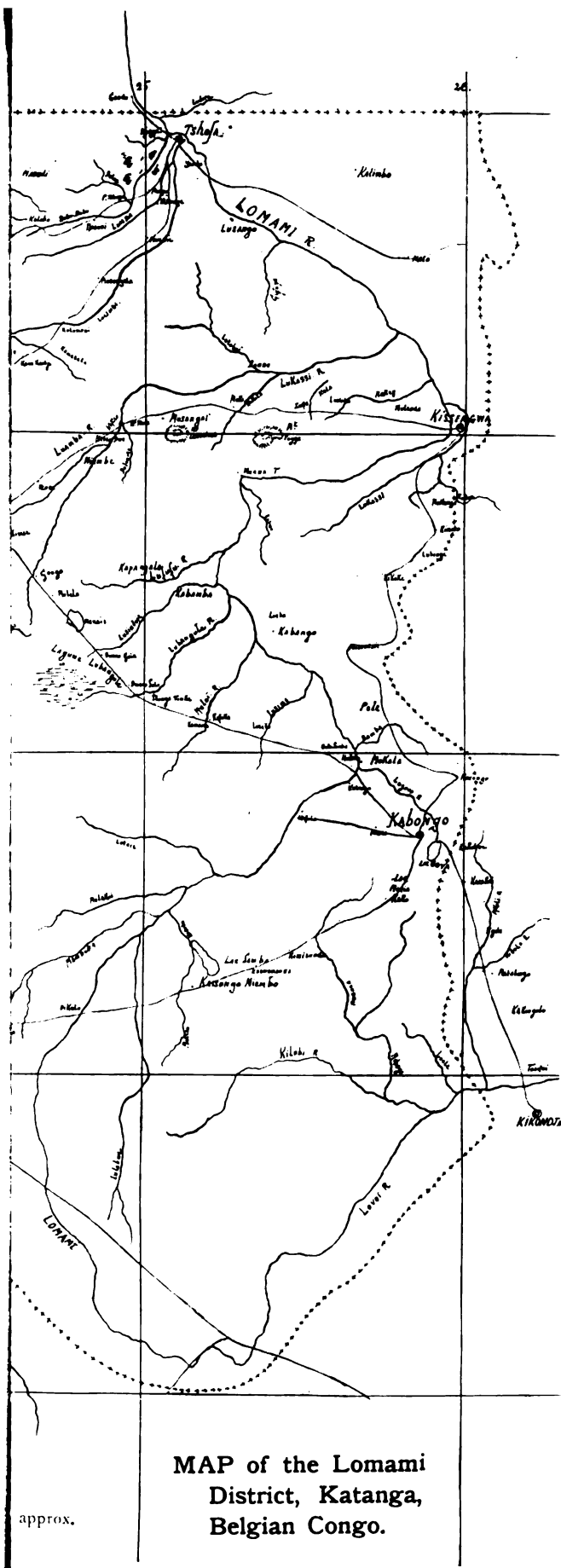
I have noticed further, at various times, certain morphological peculiarities among those specimens which I have collected. In some regions they have been uniformly and remarkably small. Dampness darkens the colour very greatly, and a moist specimen, when dying or after death, becomes very dark and the abdomen almost black, so that the segments can scarcely be discerned. When the abdomen of the fly is distended, either by the larva or by ingested blood, the dorsum of the abdomen becomes distinctly

paler and, owing to the distension of the intersegmental membrane, appears regularly banded. Females are generally much larger than the males, and it is in the former that the distension of the abdomen with blood is most marked, as they are more voracious and are able to ingest more blood at a single meal than the males.

KABINDA,

*November, 1913.*







## A NOTE ON A TRYPANOSOME OF THE BLACK RAT (*EPIMYS RATTUS*)

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### PLATE XXXVIII

The trypanosome, of which a description follows, was found in the blood of a young black rat (*Epimys rattus*) sent to the laboratory, Accra, on August 5th, 1915, by the Medical Officer of Health, Dr. J. B. Alexander, to whom I take this opportunity of tendering my thanks.

On examining the blood of this animal immense numbers of trypanosomes were found to be present. The parasites were evidently of the *T. lewisi* type, but even at first glance it was obvious that they were extraordinarily polymorphic, and that some of the forms were very large, and characterised by a remarkable prolongation of the posterior end into a whip-like extension. The trypanosomes were very active, and when progressing moved rapidly across the field of the microscope. Their movements were of two kinds. The one kind, a large wriggling movement involving the whole body, was that seen when the parasites were progressing; the other kind was exhibited when the trypanosomes were stationary, and consisted in a very rapid vibration of the anterior end. By means of the latter movement the blood corpuscles were set rotating on their own axes, and at the same time revolving in a continuous stream round and round the anterior end of the trypanosome. The movement was such as might have been produced by the flagellum being wrapped round the corpuscles, and then suddenly withdrawn, much as the string is drawn from a whip-top; but owing to the activity of the movements it was impossible to make out how they were actually brought about.

In stained specimens the polymorphism of the parasite was seen

to be extreme. Some of the trypanosomes were very small, others very long; some slender, others broad. A hundred individuals, taken as they came, were drawn with the camera lucida, and measured by the tangent line method. The longest of these measured  $48\mu$ , the shortest  $15\mu$ , and the average length was  $30.02\mu$  (see Tables 1 and 2). The majority, fifty-five, were between  $30\mu$  and  $39\mu$  in length. In breadth the trypanosomes averaged  $2.62\mu$ , and ranged from  $2\mu$  to  $6\mu$ ; but the great majority were about  $2\mu$  broad (see Table 3).

From such a small number of measurements it is of course impossible to plot a reliable curve representing the distribution of the trypanosome according to length. Neither are the extremes represented in these few measurements. Elsewhere in the films parasites were measured which reached a maximum of  $52\mu$  in length on the one hand, and on the other a minimum of  $12\mu$ . Some aggregates of division forms were also seen, similar to those found in *T. lewisi* (Plate XXXVIII, fig. 8).

Certain features were common to all the forms of the trypanosome. The body was in every case prolonged posteriorly for a considerable distance beyond the micronucleus; the cytoplasm was seldom or never granular; the micronucleus was large and oval or rod shaped; the nucleus was rounded or oval, and usually situated well in the anterior part of the body; the undulating membrane was but slightly developed; and there was always a well marked free portion to the flagellum. Four types of parasite could be distinguished, but between them, and linking them together, every intermediate stage could be found; and for this reason I believe that the rat was infected with only a single species of trypanosome. The majority of the trypanosomes were about  $30\mu$  to  $35\mu$  in length, and  $2\mu$  in breadth (figs. 7, 10). The cytoplasm stained a reddish-blue colour by Leishman's method, and was without definite granules. At the anterior end there was a well marked free flagellum, and the posterior extremity was prolonged for a considerable distance beyond the micronucleus. Very much smaller trypanosomes, some of which measured only  $12\mu$  in length, which stained similarly, were also fairly common (figs. 18, 16). In them the posterior end tapered rapidly into a finely pointed cone, and the micronucleus was not infrequently situated alongside of, or even slightly anterior to, the

TABLE I.—Distribution, by percentages, in respect of length of a trypanosome of the black rat.

Length in microns																
15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
1	1	0	3	4	5	0	0	3	3	2	4	3	3	8	7	8

Length in microns																
32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
6	10	6	10	5	1	1	1	0	0	1	2	0	1	0	0	1

TABLE II.—The lengths of 100 trypanosomes from a black rat distributed in groups of ten microns each.

Length in microns			
10-19	20-29	30-39	40-49
9	31	55	5

TABLE III.—Breadths, to the nearest whole numbers, of 100 trypanosomes from a black rat at Accra.

Breadth in microns				
2	3	4	5	6
63	20	11	4	2

macronucleus (fig. 15). Larger forms were also common. They were broad trypanosomes, sometimes measuring  $6\mu$  at their widest part, with cytoplasm that stained a bright blue, and showed an alveolar structure (figs. 4-6). The posterior end was prolonged beyond the level of the micronucleus as a broad-based cone, and the anterior end terminated in a well developed free flagellum. The macronucleus was placed anteriorly, the micronucleus a little to the posterior side of the middle point. There was generally a conspicuous vacuole just anterior to the micronucleus. These three types were very similar to those figured by Delanoë (1915) as typical of *T. eburneense*; the first type representing the adult, and the other two the multiplicative forms. In addition, however, in this rat examined at Accra, there were some very remarkable forms with the posterior end prolonged into a whip-like extension (figs. 1-3). These trypanosomes stained a reddish-blue colour, and measured up to  $52\mu$  in length, and  $3\mu$  or  $4\mu$  in breadth. Intermediate forms were found which seemed to link this type with the commonest form of trypanosome present in the blood. The anterior end terminated in rather a short free flagellum; the macronucleus was situated in the anterior part of the body; and the micronucleus, which was rod shaped, lay right across the posterior end at the point where the whip-like extension might be considered to begin. Beyond the micronucleus the posterior end of the body was drawn out into a fine flagellar filament measuring in some instances  $24\mu$  in length. The following are the detailed measurements of three individuals of this type:—

Free portion of the flagellum ... ..	$6\mu$	$6\mu$	$5\mu$
Anterior extremity of the body to the middle of the macronucleus ... ..	$8\mu$	$10\mu$	$10\mu$
Middle of the macronucleus to the micronucleus	$12\mu$	$10\mu$	$12\mu$
Micronucleus to the posterior extremity of the body ... ..	$21\mu$	$24\mu$	$23\mu$
Total length ... ..	$47\mu$	$50\mu$	$50\mu$
Breadth at the widest point ... ..	$3.5\mu$	$4\mu$	$2.5\mu$

Such forms are similar to those found by Lingard (1906) in *Mus niveiventer* and *M. decumanus*, for which the name *T. longo-*

*caudense* was originally proposed, but which subsequent observations have proved to be forms of *T. lewisi* which are 'of constant occurrence and very numerous at a certain stage of the multiplication-period' (Minchin, 1912).

On August 6th, the day after the rat was first examined, the forms of trypanosome found in the peripheral blood were similar to those described above; but on the following day the *T. longocaudense* forms and the very small forms were exceedingly rare. On subsequent days only the first type, trypanosomes of the adult form, was represented. At this period of the infection the trypanosomes were on the average somewhat longer than typical examples of *T. lewisi*, but the difference was of a degree that could scarcely have been appreciated without measurements. The average length of twenty-five individuals that were drawn and measured by the tangent method was  $33\mu$ .

Delanoë (1915) has recently published an interesting account of the trypanosomes found by him in the course of the examination of 600 rodents at Bouaké, on the Ivory Coast. One of the species described, that for which the name *T. eburneense* is proposed, appears to resemble closely the trypanosome described above, with the exception that none of the large forms with the whip-like extension of the posterior end seem to have been present. It is hardly possible that such remarkable forms could have been overlooked, but it is possible that in the animals examined the infection had advanced beyond the stage at which these forms are present in the blood; for *T. eburneense* is a trypanosome of the *T. lewisi* type, and showed other multiplicative forms similar to those of *T. lewisi*, and may therefore in all probability be assumed to show at times the forms with the posterior end greatly extended.

Thirty-six adult trypanosomes from naturally infected *Mus concha* were measured by Delanoë. The average length was  $34.9\mu$ , and the range from  $32\mu$  to  $38\mu$ . These measurements are similar to those of the commonest form of trypanosome found in the black rat examined at Accra, which were from  $30\mu$  to  $39\mu$  in length, and which constituted 55 per cent. of the forms present on the first day on which the rat was examined. It would appear probable therefore that the trypanosome found in the black rat at Accra was of the same species as that found in *Mus concha* by Delanoë, and described by him under the name *T. eburneense*.

The question then arises is this trypanosome sufficiently distinct to be entitled to specific rank or should it be considered as merely a variety of *T. lewisi*. Delanoë concluded that his measurements showed that the trypanosome of *Mus concha* was decidedly longer than *T. lewisi*. It may be doubted whether the measurement of only thirty-six trypanosomes at an unknown stage of the infection is sufficient evidence for such an assumption. *T. lewisi* measures, according to Laveran and Mesnil,  $24\mu$  to  $25\mu$  in length, but other observers have given somewhat higher figures, and three individuals measured by Delanoë himself were found to be  $32\mu$ ,  $32\mu$  and  $29\mu$  long respectively. Other trypanosomes are known to show marked variation in average length at different stages of the infection, and also when introduced into different hosts; and there would appear to be no reason why *T. lewisi* should be an exception to this rule.

*T. eburneense* was found in six specimens of *Mus concha*, all of which were somewhat heavily infected; and by means of inoculations the parasite was transmitted to *Golunda campaneae*, 'rats savanes,' and *Xerus erythropus*, but striped rats (rats rayés), white rats, and guinea-pigs appeared to be refractory in the few experiments that were made. The trypanosome appeared to possess some degree of pathogenicity, especially when inoculated into *Golunda campaneae*. The trypanosome found at Accra was inoculated into only one white rat, and one guinea-pig, but no infections were thus transmitted. It is difficult to decide what degree of importance should be attached to such inoculation experiments since *T. lewisi* has been transmitted to various animals besides rats, and it does not appear to be known whether *Mus concha*, and the other animals to which Delanoë transmitted *T. eburneense*, are susceptible, or what effect if any is produced on the morphology of this trypanosome when introduced into such unusual hosts. It is interesting to note, however, that Brown (1914) concluded, as a result of his work on a pathogenic strain of *T. lewisi*, that 'morphological anomalies were most pronounced in infections that showed unusual conditions of multiplication and that such infections usually proved severe,' an observation that may perhaps be interpreted as supporting the view that trypanosomes of the *T. eburneense* type may be varieties of *T. lewisi*.

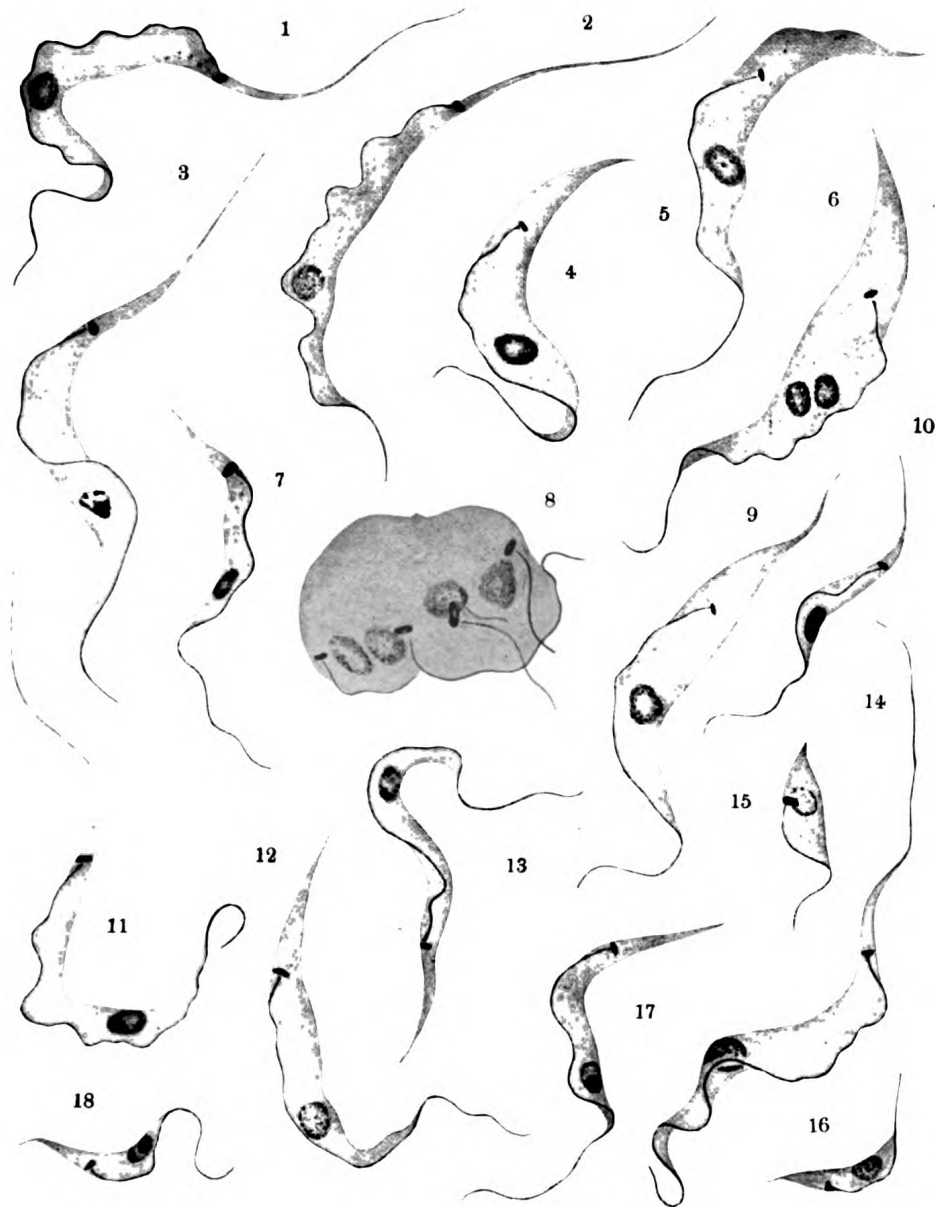


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## EXPLANATION OF PLATE XXXVIII

Figs. 1-18. Trypanosomes from a black rat (*Epimys rattus*)  
examined at Accra, Gold Coast, West Africa. × 2000.



*M. Rhodes, del.*

A TRYPANOSOME OF *EPIMYS RATTUS*.



## THE ARTIFICIAL CULTIVATION OF HANSEN'S 'BACILLUS'

BY

H. BAYON, M.D.

*(Received for publication 9 October, 1915)*

It is quite evident that a conclusive answer to the objections and criticisms of Fraser and Fletcher (see these *Annals*, July, 1915, p. 381) could only be given by discovering a method of isolating and artificially cultivating Hansen's 'bacillus,' not only with its original morphology, but also in a relatively easy fashion. No such technical refinement is known at the present moment.

A long array of negative results is no doubt a formidable argument in the hands of competent bacteriologists, but both human and rat leprosy have shown similar anomalies in relation to problems which were apparently much easier to solve than the isolation, in pure culture, of a micro-organism which belongs to a group of bacteria known to resist artificial cultivation in a most persistent fashion.

Though the communicability of leprosy has been established as a result of extensive epidemiological observations, we know that hundreds of ward attendants have worked, often for years, in asylums where hygienic precautions were practically absent and yet did not contract the disease. Therefore, following the line of argument adopted by some critics, contagion does not exist.

The long series of sterile culture tubes found by Fraser and Fletcher are not without precedent. Similar numerous attempts were carried out by K. F. Meyer before he succeeded in isolating the acid-fast micro-organism of the cattle disease which he calls enteritis hypertrophica bovis specifica, and which presents several features in common with leprosy.

If all the diphtheroids isolated from leprous nodules are contaminators, as implied by the positive assertion of Fraser and Fletcher, then surely all must show the well-known characteristics of the common *Corynebacteria* found on the body-surface. If, as many other observers contend, they are, at least in some instances, related to Hansen's 'bacillus,' then they must be distinguishable by means of sugar-tests and cultural appearances from any other similar germ. I take it, therefore that, though they do not mention the fact, Fraser and Fletcher have assured themselves, by means of the usual bacteriological methods, that the diphtheroids they isolated were identical with some well-known germ cultivated from the skin.

However, I have already acknowledged that the destructive criticism of Fraser and Fletcher can only be met by piling up experimental evidence and arraying further observations and results. At the present moment I am precluded from attempting anything in this line, but I may be allowed to point out that my last paper in these *Annals* affords an answer to one of the questions set up by my critics in a paper they published in the *Lancet* recently.

Dealing with Kedrowsky's culture, Fraser and Fletcher (1915) ask: 'Why, if this is a culture of the leprosy bacillus and it can produce leprosy in animals, did neither investigator produce similar lesions by the inoculation of emulsions of leprous tissue? Bayon, at least, had abundance of material. We have performed such experiments with uniformly negative results.'

My answer is that at Robben Island, though I had abundance of material at my disposal, I only succeeded in one single instance in producing lesions in a rabbit which lasted any length of time, in this case one year and seven months. All other experiments on rats, though I inoculated over a hundred, did not succeed to my satisfaction. In London, where I only had three cases of leprosy at my disposal, I succeeded (see Plate III, fig. 14, in these *Annals*, Vol. IX). The lesions produced are absolute counterparts of the deposits brought about in some cases by the injection of Kedrowsky's 'bacillus.' But, as stated, whether one injects ground-up nodules or Kedrowsky's culture, in the great majority of cases the bacteria get simply eliminated, without leaving any visible trace. In single rare instances, they produce bacillary deposits similar to those found in the inner organs of some lepers.

I am compelled to admit that Fraser and Fletcher are equally uncompromising when interpreting the results of their own experiments. On page 15 of the *Lancet* of July 3rd, 1915, they report on some interesting and promising injections of emulsified leprous tissue into guinea-pigs, which deserved being followed up by a sufficiently long observation of the animals, in addition to an attempt to transmit the infection to second or third generations. But the fact that not a trace of a nodule could be found anywhere after three weeks and that the organs were apparently normal, leads these investigators to the conclusion that the deposits of acid-fast rods they found in the spleen, liver, and in a peritoneal gland did not carry any conviction.

I may be allowed to say that, after having done or attended at over ninety autopsies of lepers at Robben Island, in only one single case did we find on microscopic examination acid-fast bacillary deposits in the liver resembling in quantity, intracellular and extracellular situation the well-known lesions of the skin leproma. We cannot expect skin lesions in animals inoculated with leprosy: all we can hope for are discrete deposits in the inner organs. If they can be transmitted through some generations and persist for a considerable time, and the bacillary deposit is superior to the quantity injected, then it seems to me that by all the laws of experimental medicine the inoculation has succeeded. This is the case with one experiment fully described in my paper.

It cannot too often be repeated that the scanty positive results obtained in the experimental study of leprosy are absolutely in keeping with what we know of the clinical features of the disease, its low and eminently capricious infectivity; but that here, more than when dealing with any other disease, the partial and incomplete interpretation of hundreds of negative observations cannot invalidate the proof positive of a single successful inoculation.

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# STUDIES IN BLACKWATER FEVER\*

## V.—THE DURATION OF HAEMOGLOBINURIA

BY

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*(Received for publication 28 October, 1915)*

WITH CHART

I have collected the data on this point in 167 cases. They include cases in which a relapse had occurred, the relapse being considered as a fresh attack. I have in previous studies drawn attention to the difficulty of obtaining exact records of various symptoms, and in the following analysis have only been able to classify the figures in somewhat wide intervals. As they stand, they are I think of interest, and if they are confirmed by further observations will at least tend to make our conception of the blackwater process more precise. It is possible too, that a study of the data with regard to the duration of haemoglobinuria together with a study of the temperature curves might reveal points of importance, and further a comparison with the data, if such be available, for the paroxysmal haemoglobinurias of adults and children and for the parasitic haemoglobinurias of animals might reveal points of difference or agreement which would throw light on the nature of the factors involved in the production of haemoglobinuria. At present I simply record the facts so far as I have been able to ascertain them.

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\* Part I: *Annals of Trop. Med. & Parasit.*, 1913. Part II: *Ibid.*, 1914. Part III: *Ibid.*, 1915. Part IV: *Ibid.*, 1915.

TABLE I.—Duration of Haemoglobinuria.

		Hours	Hours	Hours	Hours	Hours	Hours	Hours	Hours	Total
Duration ...	...	—	1-4	4-8	8-12	12-18	18-24	24-36	36-48	—
Cases ...	...	3*	11	9	22	9	26	22	20	122
		Days	Days	Days	Days	Days	Days	Days	Days	
Duration ...	...	—	2-3	3-4	4-5	5-6	7	8	—	—
Cases ...	...	—	30	7	5	1	1	1	—	45
										167

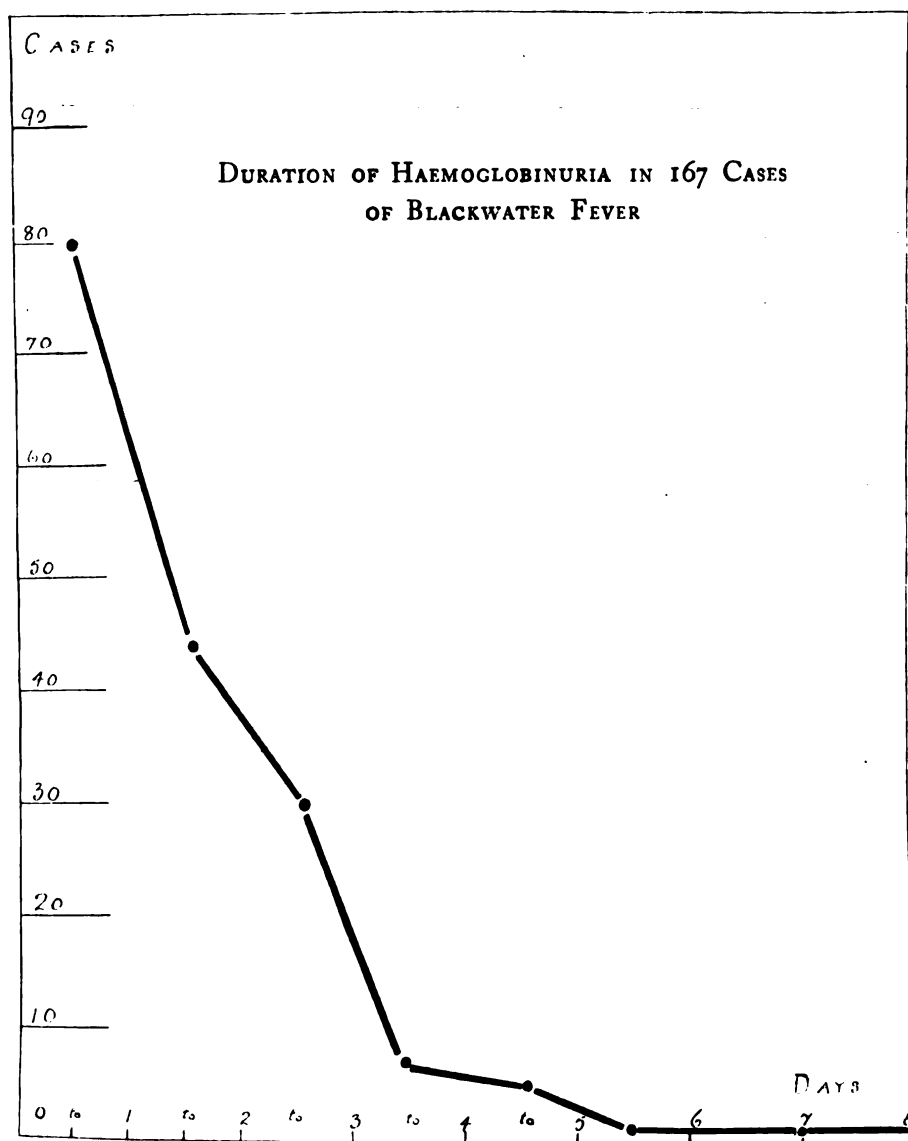
\* Haemoglobinous urine passed once only.

We may arrange these data in the following way :—

TABLE II.—Duration of Haemoglobinuria.

Duration	Cases	Per cent.
1 day or less ... ..	80	47.9
1-2 days ... ..	42	25.1
2-3 days ... ..	30	17.9
3-4 days ... ..	7	4.1
4-5 days ... ..	5	2.9
5-6 days ... ..	1	—
7 days ... ..	1	—
8 days ... ..	1	—
	167	—

In the 80 cases in which the haemoglobinuria lasted 1 day or less, the duration was 0-12 hours in 45 cases, 12-24 hours in 35.



We may also group the data into the following periods:—

TABLE III.—Duration of Haemoglobinuria.

Duration	Cases	Per cent.
Not more than 2 days ... ..	122	73·06
More than 2 days ... ..	45	26·94
Not more than 1 day ... ..	80	47·9
More than one day ... ..	87	52·1
Not more than 12 hours ... ..	45	26·94
More than 12 hours ... ..	122	73·06

Approximately, therefore, in these 167 records,

The duration is not more than 12 hours in a quarter of the cases.

„ „ „ 1 day in half of the cases.

„ „ „ 2 days in three quarters of the cases.

# SOME EXPERIMENTAL RESEARCHES ON INDUCED HERPETOMONIASIS IN BIRDS

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## PLATE XXXIX

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### 1. INTRODUCTION

The significance of certain insects in relation to disease has been recognised for some time past. That plague is conveyed by fleas, relapsing fever by lice, malaria by mosquitos, and sleeping sickness by tsetse-flies, has become common knowledge. Many insects other than the above serve as hosts for various protozoal parasites, but the latter are usually regarded as innocuous to vertebrates, more especially if the insect host is non-sanguivorous. There are,

however, a number of obscure diseases of which the excitants have not been determined, and also a number of flagellates normally parasitic in insects whose pathogenicity towards vertebrates has never been put to the test.

In continuation of our work on the introduction of insect flagellates into vertebrates, we have, for some time past, been testing the pathogenicity of certain insect flagellates with respect to birds, the last great group of the European vertebrates that has remained untested by us. The present paper records the successful infection of birds with two insect flagellates, *Herpetomonas jaculum*, Léger, from the water scorpion, *Nepa cinerea*, and *H. culicis*, Novy, MacNeal and Torrey, from *Culex pipiens*. The testing of the pathogenicity of insect flagellates towards birds has been of interest for two reasons. First, in May, 1907, Drs. Edm. and Et. Sergent recorded briefly and figured a herpetomonad that they had found occurring naturally in the blood of a pigeon in Algeria. In the second place numbers of birds die annually from other than old age and wounds, and the cause of death is never ascertained. Some of our experiments, coupled with the fact that the crops of the birds frequently contain insects, suggest that undetected herpetomoniasis may be the cause.

We have referred in our previous papers to the work of Laveran and Franchini on the introduction of insect flagellates into mammals. Our own experiments were commenced early in 1911 with rats, which were refractory to the introduced herpetomonads. However, our experiments were continued, different hosts being used with more successful results. We have much pleasure in thanking Professor G. H. F. Nuttall, F.R.S., for his kind interest in our researches, and for looking at many of our preparations.

## II. MATERIAL AND METHODS

The birds used were canaries (*Serinus canarius*), sparrows (*Passer domesticus*), and martins (*Chelidon urbica*). The canaries were bought from a bird-dealer, the sparrows and martins were caught, and were practically tame when used for feeding or inoculation. The control birds in each case are still alive and healthy, or were found healthy when killed.

The birds were fed either with the entire insects (containing herpetomonads) or with their alimentary canals that had been removed. There was usually no difficulty over the feeding. After the infective feed had been given, grain and shredded cooked meat or egg were given as food. Infection of birds by inoculation was also tried.

The insects (*Nepa cinerea* and *Culex pipiens*) were obtained chiefly from the neighbourhood of Cambridge, and some of the *Culex* larvae were bred out and identified. For the present we retain the species names of the various herpetomonads (e.g. *H. culicis* and *H. jaculum*) as given originally, though we have much evidence that such are not true species, but are rather physiological races of one or two distinct species.

Blood smears of the experimental birds were taken at intervals. Smear preparations of the organs were made at death, fixed wet with osmic vapour followed by absolute alcohol, or with Bouin's fluid, and stained with Giemsa's solution or iron-haematoxylin.

The birds used were carefully examined for ectoparasites, but none was found. Blood examinations for haematozoa at the commencement of the experiments were equally negative.

### III. EXPERIMENTAL WORK

*Herpetomonas jaculum* has been shown by us to be capable of infecting certain fish, amphibia, snakes and mice. It was therefore tested with birds. The use of *H. culicis* was suggested by finding many *Culex* remains in the crops of some sparrows and martins found dead and sent to us for examination. They had been too long dead to allow of detection of blood parasites, had any been present. In the light of our experiments it is possible that such parasites may have been present.

EXPERIMENT 1 (H. B. F.). A canary, ♀, was fed with material containing *Herpetomonas jaculum*. The bird snapped the dissected intestines of two *Nepa cinerea* containing *H. jaculum* somewhat greedily, and also swallowed two infected nymphs. It was then fed on ordinary mixed bird seed and chopped egg until the day of its death, which occurred 51 days later. Blood smears were made at intervals during the period of the experiment. At first the bird seemed quite healthy. Twenty-one days after the commencement

of the experiment, a blood smear showed the presence of elongating, post-flagellate forms of *H. jaculum*. About a fortnight later the bird became very mopy, sat huddled up, refused food and shivered. Again parasites were found in the blood. The attack lasted three days, when the bird seemed to recover. Nine days later, on the 50th day of the experiment, the bird became mopy again. On the 51st day it seemed better at first, refused its seeds, but ate the egg supplied it. During the day it was brighter again, but in the evening again refused food. It was hopping about half an hour before its death, which took place the same evening quite suddenly and with no struggle of any sort.

At the commencement of the experiment the bird weighed 26 grams; at its death it weighed 10·2 grams.

At post-mortem, the body was found to be much emaciated. The liver seemed normal, the spleen slightly enlarged. Only a very small quantity of bone-marrow was present. The suprarenal bodies were very hard and firm, much more so than in normal birds. Smears were made of all the organs. When examined the results were as follows:—Leishmania-like bodies and some uninucleate forms were found in the liver, which also contained a few elongating parasites and still fewer typical, flagellate herpetomonads. The spleen smear showed a few uninucleate and leishmaniform organisms, some of them intracellular. The heart and lungs harboured leishmaniform bodies, and in the lungs developing forms showing the root of the flagellum were seen. Smears of the kidney showed the presence of a few uninucleate forms and some leishmania-like parasites, and similar bodies were present in the bone marrow. No parasites were found in the suprarenal bodies.

A control bird was killed, but no parasites were found in its organs, nor had it lost weight.

EXPERIMENT 2 (A.P.). This experiment was undertaken as an examination of the crop contents of several sparrows found dead had shown the presence of a number of the common gnats, *Culex pipiens*. These gnats are known to harbour *Herpetomonas culicis*. A number of larvae and of adults were obtained, and were fed to a ♀ sparrow, which was fairly tame. The insects were taken by the bird without any trouble. Four days later the bird became mopy and had a shivering attack, which soon passed off. A fresh



blood film showed a single non-flagellate herpetomonad. On the 9th day of the experiment the bird died suddenly. The liver and spleen were softish, the kidneys, suprarenal bodies and lungs seemed normal. The bone-marrow was small in quantity, but more fluid than usual. The pancreas appeared to be enlarged. The ovary was large and contained numerous ova. No protozoal parasites were found post-mortem in blood examined fresh or in the intestine. The bird weighed 22·7 grams at death, showing a loss of 2·2 grams from the commencement of the experiment, and exhibiting some emaciation.

At death, smears were made of the organs. The stained smears showed that, as in the case of the canary, there was a generalised infection of the parasite, in this case, *Herpetomonas culicis*. The flagellate form of the herpetomonad predominated here. The non-flagellate stages were less common. Transitional forms also were present. The heart, liver, lungs, kidneys, and suprarenal bodies contained well-developed flagellates. Leishmaniform bodies were also present in smears of these organs, but were less frequent than the flagellate forms. The bone-marrow also contained them as well as elongating parasites. Leishmaniform elements in process of division were found in the heart and liver, dividing elongating but non-flagellate herpetomonads occurred in the bone-marrow, while fully developed flagellates in various stages of division were present in smears of the heart, liver and lungs.

Blood smears taken during the course of the experiment, and stained, showed no parasites except on the day before death. A smear taken 14 hours before death contained a single leishmaniform element in process of elongation. The blood showed signs of anaemia, and was very fluid.

The control sparrow is still alive and active.

EXPERIMENT 3 (H.B.F.). A young adult ♂ martin was fed with larvae and mature *Culex pipiens* containing *Herpetomonas culicis*. It lived twelve days after the infective feed. The condition of the bird at death and the distribution of the parasites was practically the same as that of the sparrow, given in Experiment 2, so that the description need not be repeated. Blood smears taken during the course of the experiment were negative.

EXPERIMENT 4 (A.P.). A young adult ♀ martin was inoculated subcutaneously with *H. culicis*. The bird was greatly frightened

and died after two days. No infection appeared to have taken place.

EXPERIMENT 5 (A. P.). A young ♂ canary was fed with the faeces of *Nepa cinerea*, the excrement, which contained *H. jaculum*, having been collected on a slide and mixed with bread. Blood examinations were made at intervals. The bird lived 17 days after the infective feed. A few non-flagellate forms were found on the 7th and 11th days. At post-mortem, a few non-flagellate forms were found in the liver and spleen, elongating parasites in the liver and bone marrow, and a few flagellates in the liver. The heart-blood and tissue contained some multiplicative forms.

EXPERIMENT 6 (H. B. F.). A young mature martin, ♂, was fed with the faeces of several larvae and adults of *Culex pipiens*, mixed with small quantities of boiled mutton. The *Culex* faeces contained post-flagellate or encysted stages of *H. culicis*. The bird lived 32 days after the infective feed. The body was somewhat emaciated at death. Non-flagellate forms of *H. culicis* were present in the spleen and lung, elongating forms in the bone-marrow and a very few flagellate stages in the spleen.

EXPERIMENT 7 (H. B. F.). A ♀ sparrow was fed with the faeces of *Nepa cinerea* containing *H. jaculum*. On the 11th day after the infective feed, a probable parasite of the elongating flagellate type was seen in the blood, but no others have been observed since. The bird is growing somewhat thinner but is still alive at the time of writing, and infection appears doubtful.

EXPERIMENT 8 (A. P.). A ♀ canary was fed with food contaminated with *H. culicis*. As usual, blood smears were taken at intervals. No parasites were found. After 80 days, the bird was killed, but no herpetomonads were found on examination of organ smears.

In connection with these experiments, it should be remembered that the flagellates of the insect hosts rarely co-existed with many bacteria. In common with certain other workers, we found that when the insects contained many bacteria, the protozoal flagellates usually disappeared. Further, some digestion experiments performed by us have shown that many bacteria introduced into the digestive fluids of the bird's stomach are destroyed by the same, while the flagellates are but little affected. This is not surprising, since certain protozoal infections of man are known to flourish in an acid medium, and are combated by the use of alkaline substances.

#### IV. THE MORPHOLOGY OF THE PARASITES IN THE INSECT AND THE AVIAN HOSTS

The morphology of *Herpetomonas jaculum* and *H. culicis* in the respective insects and birds is of interest. Little morphological difference can be found in either case. It may be noted that when the infection was of the chronic type, as in the canary (Expt. 1), the non-flagellate, leishmaniform bodies preponderated in the smears of the organs, while the mature flagellates were more numerous in the cases of the sparrow and martin (Expts. 2, 3), where the herpetomoniasis was of the acute type. We would point out that while such was the case in these experiments of ours, we do not consider that any generalisation can yet be made therefrom. However, it may be noted that Monge (1914), dealing with flagellate stages of *Leishmania tropica* in man in Peru, states that the presence of such flagellate stages may be an indication of increased virulence. More experiments are needed, and some are now in progress.

##### A. *Herpetomonas jaculum* IN THE NEPA AND THE CANARY

*Herpetomonas jaculum* parasitic in *Nepa cinerea* shows much variation in size. The non-flagellate stages are oval, and show a nucleus and well marked blepharoplast. The position of the blepharoplast varies. These non-flagellate forms elongate, the extension often being preceded by division of the nucleus and blepharoplast. The root of the flagellum differentiates and finally reaches the exterior, forming the free flagellum. The body also elongates, and the mature flagellate is thus produced. When change of host is necessary, retrogression and absorption of the flagellum occurs, the body concentrates, and a cyst wall, at first gelatinous but later shrinking to a thin, varnish-like coat, is produced. This is the post-flagellate or encysted form of the parasite, adapted for life outside the body of the host. When ingested by a fresh host, it becomes the leishmaniform, pre-flagellate organism with which the cycle commenced.

*Herpetomonas jaculum*, as found in the experimentally infected canary (Expt. 1), showed non-flagellate and flagellate forms, in various stages of growth and division. Non-flagellate forms (Plate XXXIX, figs. 1-15) were often from  $4\mu$  to  $6.6\mu$  long by  $2\mu$  to  $5\mu$  broad. Usually they were found singly (figs. 2-10), sometimes

clusters of two (fig. 11) were present, and on one occasion only a number of somewhat narrow forms (fig. 12) was found. The body cytoplasm was slightly alveolar. The nucleus usually was finely granular and homogeneous (figs. 3-6); more rarely a karyosome was seen (figs. 2, 7). The blepharoplast occupied different positions in the body (figs. 1-10). It was often barlike (figs. 14, 15), sometimes rounded (figs. 7, 17). No differentiation of structure was seen within it. Dividing non-flagellate parasites were not numerous.

The elongating herpetomonads presented much the same structure. In some of them the root of the flagellum was seen (figs. 16, 17). This structure, as mentioned by one of us in a previous paper, was first described by Mesnil and colleagues (1904) for *Leishmania tropica*, by Christophers (1904) for *L. donovani* and by Novy (1909) for *L. infantum*.

Full-grown flagellates (figs. 18, 19) were relatively rare in the canary at the time of its death. It is possible that they may have been more numerous at some period of its life, but that could not be ascertained, the organisms usually occurring in the internal organs of the host. Morphologically, the flagellates were like those in the insect host, but the maximum size, as in previous experiments, was not attained, though a flagellate was found with a body-length of  $20\mu$ .

From the foregoing it is seen that there is a close resemblance between *H. jaculum* in the insect host and in the avian host into which it was introduced by feeding, while the non-flagellate forms recall those of the various leishmaniasis of man. Further statements regarding this subject will be made later.

#### B. *Herpetomonas culicis* IN THE CULEX, SPARROW AND MARTIN

The life-cycle of *Herpetomonas culicis* in *Culex spp.* resembles that of *H. jaculum* morphologically, and need not be recapitulated here. As found in the avian hosts, the non-flagellate forms of *H. culicis* were oval or pyriform bodies (figs. 20-25), usually measuring  $4\mu$  to  $6\mu$  by  $2\mu$  to  $4\mu$ . Occasionally, rarer, larger forms were encountered (fig. 26), but it is possible that they were about to divide. Non-flagellates in various stages of division were found (figs. 27, 28).

The flagellates were elongate (figs. 30-38), with both ends more or less rounded, though the posterior end was often the more pointed. The body length usually varied from  $11\mu$  to  $16\mu$ , and the breadth from  $1.5\mu$  to  $3.6\mu$ . The flagellum was often as long or longer than the body. Still longer parasites (fig. 38) might be found. Sometimes forms about to divide were wider (fig. 29), as would be expected.

Multiplication of non-flagellate forms by binary fission was found in preparations of the heart and bone-marrow, while dividing flagellates (figs. 39, 40) were present in the smears of the heart and lung. Rosettes of parasites, produced by repeated binary fission in the insect host, were not found in the bird smears.

The nucleus of *H. culicis* was round (figs. 22, 33) or oval (figs. 25, 31, 36), and was most frequently granular (figs. 21-32). Occasionally a nucleus showing a karyosome was encountered (fig. 33). The blepharoplast was always conspicuous, and varied from round (figs. 31, 36-38) to bar-like (figs. 34, 35). Metachromatic granules (fig. 35) were not common in the cytoplasm, which was alveolar. Traces of contractile myonemes (fig. 33) were occasionally seen.

The morphology of the parasite in the vertebrate host, then, is practically identical with that of the Protozoön in the gnat. The plasticity of the organism is apparent, and the capacity for morphological variation in the parasite coincides with the increase in its pathogenicity to its new hosts.

The results of the foregoing experimental work show that *Herpetomonas jaculum* and *H. culicis* can parasitise and be pathogenic to birds to which they gain access. The adaptability of the flagellates to life in vertebrate hosts is well marked. They exhibit the same morphology as when in the insect host, and attain almost the same dimensions. The degree of pathogenicity induced appears to vary, the disease resulting being of a chronic or of an acute type. In the chronic type of infection in birds, the leishmaniform, non-flagellate phase of the herpetomonad was the more obvious, while in the more acute cases, a greater number of flagellates was present. Monge (1914) made suggestions of a similar import in regard to *Leishmania tropica* in man in Peru. The parasites are much more numerous in the internal organs of the experimental birds than in the circulating blood, a feature common in kala-azar.

## V. NATURAL HERPETOMONIASIS IN BIRDS

As before mentioned, in May, 1907, a short account of a herpetomonad occurring in the blood of a pigeon was given by Drs. Edm. and Et. Sergent. During their studies of the Haemosporidia of birds, when working on the relations of *Haemoproteus columbae* in pigeons and in the second host which they discovered to be the Hippoboscid fly, *Lynchia maura*, they found a herpetomonad in the blood of one of their Parisian pigeons. The body of the herpetomonad was straight and drawn out, measuring  $17\mu$  to  $22\mu$  by  $1.5\mu$ . The flagellum measured  $19\mu$  to  $35\mu$ . No undulating membrane was present. The elongate nucleus, measuring  $5\mu$  to  $7\mu$  in length, was not as wide as the body, and was situated  $6\mu$  to  $7\mu$  from the posterior extremity of the body. The large, spherical, heavily staining blepharoplast was  $3\mu$  to  $5\mu$  in front of the nucleus. Care was taken by the authors to avoid confusion with spermatozoa of the bird. Two excellent figures of this herpetomonad were given. The parasite has not been observed since. It is much to be regretted that an opportunity for following further this interesting discovery did not arise.

The source of the herpetomonad is not known with certainty. The bird containing the parasite had been inoculated intravenously with part of the Berkefeld filtrate of an emulsion of ten *Lynchia maura* previously fed on a pigeon infected with *Haemoproteus columbae*. As the bird contracted the Haemoproteus infection, it seems possible that this filtrate\* also furnished the flagellate, or the bird may have had a latent herpetomoniasis contracted direct from insect hosts.

Considerable resemblances are presented by the account of this presumed natural herpetomoniasis of the pigeon and the induced herpetomoniasis in our birds—canary, sparrow and martin. The flagellate form of the parasite is well marked in each host. The free flagellum is distinct, the nucleus and blepharoplast have the same staining reactions. The same parallel holds in the case of the natural and induced herpetomoniasis of mice recently described by us. These parallel conditions suggest that these diseases induced

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\* Since this was written, Dr. Edmond Sergent has kindly informed us that so far as he was aware, his *Lynchia* were not infected with herpetomonads. Our second hypothesis, then, may be the correct one. We wish to thank Dr. Sergent for his courtesy and great interest.

by natural flagellates of invertebrates will, sooner or later, be found in parallel under conditions not those of the laboratory. Some, in fact, have already accrued (see Section VI).

#### VI. THE FLAGELLATE STAGE IN HERPETOMONIASIS AND LEISHMANIASIS

Recently one of us pointed out in these *Annals* that the flagellate form of *Leishmania* had been known for some time in man, while it had been known for considerably longer in cultures. Briefly, Escamel, in 1911, in Peru saw flagellate forms of *Leishmania tropica* in man, and published about them later. La Cava (1912) described similar forms of the same parasite in Italy. In 1912 Splendore found elongating forms and a few flagellate parasites in cases of dermo-mucosal leishmaniasis in Brazil, while Monge (1914) found the herpetomonad stage of the parasite of the same malady in Peru. The *Leishmania* cycle, then, is that of the herpetomonad.

There was thus considerable evidence of the existence of the flagellate stage of *Leishmania* in the vertebrate host at the time when we suggested, as Patton has also done, that canine kala-azar was really a herpetomoniasis due to *H. ctenocephali*, that leishmaniasis were really arthropod-borne herpetomoniasis, and that it was 'likely that certain vertebrates . . . especially those that are insectivorous, may serve as reservoirs for leishmaniasis,' these conclusions having resulted from our former series of experiments. We also stated that 'in areas where leishmaniasis are endemic, an examination should be made of all insects and other invertebrates likely to come into contact with men or dogs or rats and mice, in order to ascertain if these invertebrates harbour herpetomonads. Preventive measures should be directed against such invertebrates, especially arthropods,' Additional support to the belief that leishmaniasis are really herpetomoniasis has been received quite recently by the announcement that in September, 1915, Wenyon has found the flagellate stage of *Leishmania donovani* in a dog subinoculated from other dogs, the strain being derived from a man who died of kala-azar contracted in Calcutta. He has also found variation in

the size of the Leishman-Donovan bodies in animals, as has been previously noted by us in the non-flagellate stages of herpetomonads introduced into vertebrates.

As we have shown elsewhere, flagellate as well as non-flagellate forms of the parasites occurred in the internal organs of sticklebacks, frogs, toads, snakes, mice and dogs, infected with *Herpetomonas jaculum*, *H. stratiomyiae*, *H. ctenocephali*, or *H. pediculi*. To this list of hosts we can now add the canary, sparrow and martin, and *H. culicis* increases the list of parasites. Comparing these experimental results with the finding of the flagellate stages of *Leishmania donovani* and of *L. tropica* in dogs and in man, respectively, the evidence that the parasites of leishmaniasis are really herpetomonads (or leptomonads, as some authors term them) seems thus conclusive.

It is not always easy to find flagellate forms of the various herpetomonads that we have used. They are fragile, and are easily broken, and disintegrate relatively soon after the death of the host. Again, the flagellate stage in the development of the parasite is not always present at the time of death. It is probably owing to these two factors that the flagellate *L. donovani* has not been found direct in man so far.

Recently one of us drew attention to the flagellate forms described by Darling in connection with *Histoplasma capsulatum*. It seems probable that the parasites in question may have been those of a herpetomonad co-existing with rounded *H. capsulatum*, considered by many authorities to be a yeast. Up to the present, no explanation of the significance of these flagellate forms has been afforded, and before final classification of the organism can be made, it will be necessary to elucidate whether these elements are independent or have a connection with *Histoplasma*. Mixed infections of herpetomonads and trypanosomes are capable of co-existence, and this should also be remembered when so-called 'herpetomonad phases of trypanosomes' are found.

The accumulation of evidence regarding the existence of a flagellate stage in natural and induced herpetomoniasis in vertebrates shows the necessity for considering not part but the whole of the life-history of an organism, and not only that, but the relation of the parasite to the group to which it belongs. There is a line of evolution common to each group, and in these cases, neither



*Herpetomonas* (*Leptomonas*), *Leishmania* nor *Trypanosoma* should be considered as isolated units, but as flagellates belonging to the Trypanosomidae. Much of the discussion that has arisen in latter years would have been unnecessary were the organisms considered from the broader, comparative standpoint.

## VII. GENERAL CONCLUSIONS

The general conclusions that have resulted from our series of experiments are as follows:—Under suitable conditions, insect flagellates can be introduced into vertebrate hosts and produce infection therein. In some cases, as in cold-blooded vertebrates, little obvious ill-effect results; in others, as in mammals and birds, disease is manifested and often ends in death. Similar infections are known to occur naturally in some cases, for example, in mice and pigeons.

The organisms, such as herpetomonads, thus introduced, retain their powers of development on the same lines as when they were present in the insects. The morphological cycle of *Leishmania* is like that of *Herpetomonas*. The various species of *Leishmania* are probably insect herpetomonads long since introduced into man and usually perpetuating the non-flagellate, relatively more resistant form, though capable of assuming the flagellate, herpetomonad facies in the internal organs of the vertebrate or in the invertebrate host.

Various vertebrates—fish, amphibia, reptiles, birds and mammals—may serve as reservoirs of leishmaniasis. The virus may be very attenuated and so escape detection, or only be revealed by the presence of the flagellate forms in cultures. It has also been suggested by Stephens (1915) that each case of leishmaniasis in vertebrates arises *de novo* from the introduction of insect flagellates.

No insect flagellate can be considered to be quite innocuous to vertebrates until it has been put to the test.

Leishmaniasis, which is a form of herpetomoniasis (leptomoniasis), is a flagellosis, as is also trypanosomiasis. Treatment of leishmaniasis by intravenous injections of tartar emtic, as advocated and practised recently by Vianna, Carini, di Cristina and Caronia, Rogers and others, is sound biologically, as drugs containing arsenic or antimony have proved efficacious in trypanosomiasis. It is thus

seen that the researches of the comparative morphologist are of the greatest possible assistance to the medical man when founding a basis for therapeutics.

### VIII. SUMMARY

1. Herpetomoniasis can be induced in birds, for example, canaries (*Serinus canarius*), sparrows (*Passer domesticus*) and martins (*Chelidon urbica*), by feeding them on insects containing herpetomonads.

2. *Herpetomonas culicis* from *Culex pipiens* and *H. jaculum* from *Nepa cinerea* have fatally infected birds when fed to them. Both flagellate and non-flagellate herpetomonads have been found in the internal organs of the infected host.

3. The cycle of the flagellates in the avian hosts resembled morphologically that in the insects.

4. The disease induced may run an acute or a chronic course. In the acute cases in our birds the flagellate form of the parasite was the more obvious at death. In chronic cases, non-flagellate forms of the parasite were more numerous.

5. Natural herpetomoniasis of a pigeon has been recorded by Drs. Edm. and Et. Sargent in Algeria. This affords a parallel case with the natural and induced herpetomoniasis in mice previously recorded by us.

6. The flagellate stage of *Leishmania donovani* in vertebrates is now known, and that of *L. tropica* in man has been known for some time. The links completing the evidence that a *Leishmania* is morphologically a *Herpetomonas* are thus complete. Leishmaniasis are really herpetomoniasis (or leptomoniasis) arising from herpetomonads of certain invertebrates.

7. Members of all classes of vertebrates may be capable of acting as reservoirs of herpetomoniasis, and the virus may exist in a very attenuated condition and so be difficult of detection.

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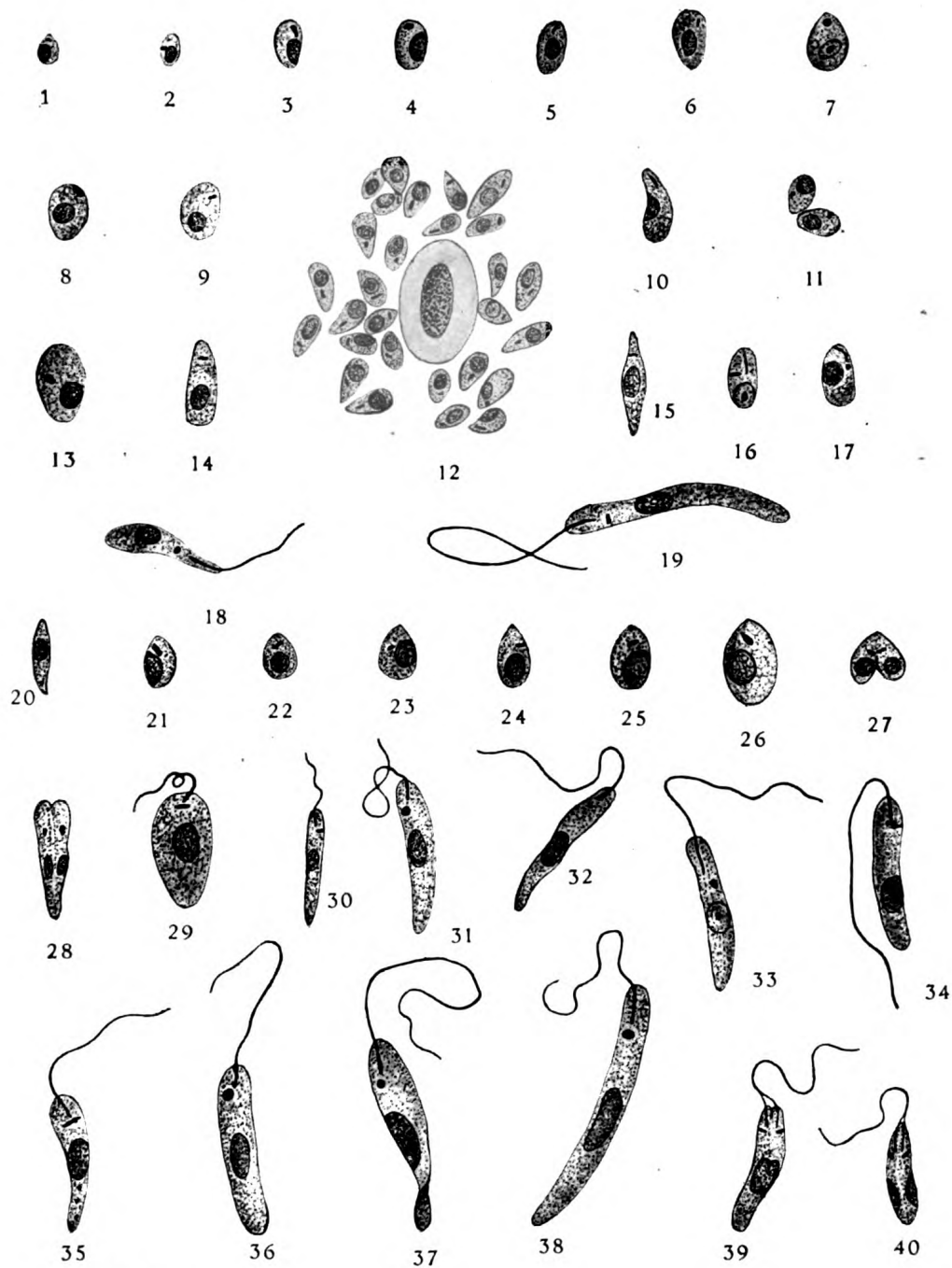
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## EXPLANATION OF PLATE XXXIX

All figures were outlined with an Abbé-Zeiss camera lucida, using a Zeiss  $\frac{1}{8}$  in. objective and compensating ocular 8. The magnification is approximately 1,500 diameters.

- Figs. 1-19. *Herpetomonas jaculum*, from the canary.
- Figs. 1-15. Non-flagellate forms of *H. jaculum*.
- Fig. 1. Small parasite. From circulating blood.
- Figs. 2, 9. Free forms. Heart smear.
- Figs. 3, 5. Leishmaniform parasites. Bone-marrow.
- Figs. 4, 8. Non-flagellate forms. Lung.
- Figs. 6, 7. Parasites from kidney. Fig. 7 shows rounded blepharoplast and karyosomatic nucleus.
- Fig. 10. Slightly curved parasite. Spleen.
- Fig. 11. Group of two pyriform parasites. Blood.
- Fig. 12. Group of small non-flagellate parasites around an erythrocyte. Such groups are most uncommon. Blood.
- Figs. 13, 14. Large parasites. Liver.
- Fig. 15. Elongating *H. jaculum*. Blood.
- Figs. 16, 17. Parasites showing root of flagellum. Fig. 16 shows a karyosomatic nucleus. Lung.
- Figs. 18, 19. Flagellate forms of *H. jaculum*. Liver.
- Figs. 20-40. *Herpetomonas culicis*, from the sparrow and martin. Figs. 22, 23, 25, 30, 37, 39 from martin; the rest from the sparrow.
- Figs. 20-26. Non-flagellate *H. culicis*. The parasite in Fig. 20 has the blepharoplast superimposed on the nucleus. Figs. 20, 21, from blood; Fig. 22, from bone-marrow; Fig. 23, from lung; Fig. 24, from liver; Figs. 25, 26, from heart smears.
- Fig. 26. Larger non-flagellate parasite.
- Figs. 27, 28. Two dividing parasites, from heart and bone-marrow respectively.
- Figs. 29-40. Flagellate *Herpetomonas culicis*.
- Fig. 29. Very stout flagellate, probably about to divide. Heart.
- Figs. 30, 31. Flagellates from liver smears.
- Fig. 32. Parasite from kidney smear.
- Fig. 33. Flagellate with karyosomatic nucleus. Heart.
- Fig. 34. Flagellate from suprarenal capsule.
- Fig. 35. Parasite from heart, showing a few metachromatic granules.
- Fig. 36. Stout form from the lung. Round blepharoplast.
- Fig. 37. Flagellate folded on itself, as is frequent in life. Liver.
- Fig. 38. Elongate flagellate. Liver.
- Figs. 39, 40. Dividing flagellates from the hearts of the martin and sparrow respectively.



A. P. et H. B. F. del.

# INDUCED HERPETOMONIASIS IN BIRDS.

FIGS. 1—19. *Herpetomonas jaculum* in Canary.  
 FIGS. 20—40. *H. culicis* in Sparrow and Martin.



ON THE ASSOCIATION OF  
WARTHOG AND THE NKUFU TICK  
(*ORNITHODORUS MOUBATA*)

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(Received for publication 12 November, 1915)

The following observation was made during searches for the pupae of *Glossina morsitans* near Hargreaves, in the lower part of the Luangwa Valley, N. Rhodesia. A native was sent into a warthog burrow, in which the pupae are not infrequently found, to scrape out the loose earth for examination. He was at once attacked by a number of nkufu ticks with which the burrow was infested. About thirty were removed from his clothing, and especially from his head where they were actively feeding. Most of the ticks were very young larvae which had not previously fed, some were nearly full grown, and intermediate stages were represented. No adults were seen.

The observation is of additional interest as regards the locality. At the time that suggestions were being made as to possible carriers of *Trypanosoma rhodesiense*, the nkufu tick, among other blood suckers, fell under suspicion. It was pointed out, however, that it was not generally distributed in the Luangwa Valley, the only locality where it was known to occur being the compound of the rubber plantation at Hargreaves (Neave). This plantation has now been deserted for five years. The burrow in which the ticks were found was on the opposite bank of the river, and the nearest village was Mwapi, four miles away, where the tick is said not to occur.

Mr. L. C. Heath (N.C. at Mwengwa, N. Rhodesia) informed the writer that he had removed a tick from a warthog which was identified at Cambridge as *Ornithodoros moubata*. The warthog is possibly of importance as a distributing agent for this pest, and should not be overlooked in any prophylaxis against relapsing fever.



# ON ANAPLASMA-LIKE BODIES IN THE BLOOD OF VERTEBRATES

BY

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*(Received for publication 24 November, 1915)*

TEN TEXT-FIGURES

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## INTRODUCTION

The study of vertebrate blood and its possible parasites is of great importance, and many papers have been written during the last ten years dealing with blood-parasites. Some of the papers would have been much improved by a careful preliminary study of the elements of apparently normal blood, or of blood under definite pathological conditions. A number of the so-called parasites would then have been found to be artefacts or reaction products, and not organismal. Structures probably of this nature are the Anaplasmata or 'marginal points,' for which Theiler created the genus *Anaplasma* in 1910, as he considered them to be organismal and the cause of 'gall-sickness' in cattle in South Africa. Other investigators consider Anaplasmata to be non-organismal, and to result from haemolytic conditions of the blood.

## MATERIAL AND METHODS

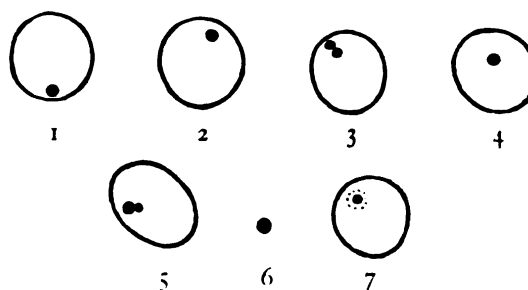
The material examined during these observations consists more particularly of mice, canaries, swallows, martins, lizards, snakes, frogs, toads and sticklebacks—that is, representatives of all the great groups of the Vertebrata. The animals were used by me, in collaboration with Dr. H. B. Fantham, on our researches into the

experimental introduction of insect flagellates—belonging to the genera *Herpetomonas* and *Crithidia*—into different vertebrates. The experiments resulted in induced herpetomoniasis in the vertebrates, often with pathogenic effects closely resembling those due to leishmaniasis. The results of these experiments have been published during 1914-15 in a series of memoirs listed in the References to this paper. The presence of Anaplasmata was noted in the blood of the experimental animals, many of which appeared to be anaemic. The morphology, distribution and significance of the Anaplasmata seen in the animals suffering from flagellosis will be considered.

Films of natural anaplasmosis in South African cattle have been used for comparison, and examinations of blood films from obscure cases of human anaemia have also been made.

### MORPHOLOGY

Anaplasmata, when seen fresh, appear as small, rounded granules or globules within the erythrocytes of the host. They show no morphological differentiation. Usually they occur singly (figs. 1, 2, 4), but forms apparently dividing and others simulating rosette formation have been found, though such may be really aggregations. When intra-vitam staining was employed, no differentiated structures were observed within the globules.



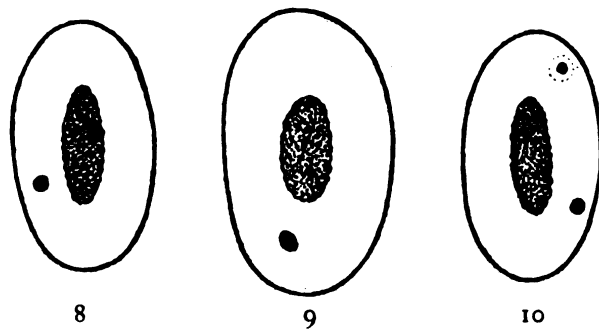
FIGS. 1 to 7. Anaplasma-like bodies from the blood of mammals, such as young mice.  $\times 1500$ .  
 FIGS. 1 and 2. Bodies like *Anaplasma marginale*.  
 FIG. 4. Similar body like *Anaplasma centrale*.  
 FIGS. 3 and 5. Red corpuscles each containing two such bodies.  
 FIG. 6. Free form.  
 FIG. 7. Marginal point surrounded by a relatively clear area.

Stained preparations showed much the same features as fresh ones. The structures always consisted of small, uniform, usually spherical masses that stained intensely with chromatin stains, that

is, they were basophilic. Sometimes the bodies were oval in shape, and only very rarely were they irregular. Very occasionally a somewhat less densely staining portion of cytoplasm surrounding the chromatinic bodies was observed (figs. 7, 10), but no marked exterior limit to such an area or halo could be found, and the staining appeared to be merely an idiosyncrasy of that portion of the host cell. It may be mentioned that the Anaplasmata showed the same type of structure whatever was the host from which they were obtained—man, cattle, mice, birds, reptiles, amphibia or fish.

The Anaplasmata measure  $0.3\mu$  to  $2\mu$  in diameter, the latter size being somewhat uncommon. Structures about  $0.5\mu$  in diameter are relatively numerous. Free forms may occur (fig. 6).

Sometimes two Anaplasmata are found apposed, a diplococcus-like structure resulting. The two individuals may be equal (fig. 3) or sub-equal (fig. 5), and appear to be the result of division.



FIGS. 8 to 10. Anaplasma-like bodies in the red blood corpuscles of cold-blooded vertebrates such as snakes and frogs.  $\times 1500$ .

FIGS. 8 and 10. Red corpuscles of grass snake containing one and two Anaplasmata respectively. FIG. 9. Red blood corpuscle of frog containing oval Anaplasma.

*Apparent multiplicative forms.* Bodies that formerly would have been considered to be of a multiplicative nature (figs. 3, 5) have been observed by me in a number of cases of anaemia due to maladies such as herpetomoniasis. The process of formation of Anaplasmata and of their pseudo-multiplication is as follows in nucleated red cells, such as those of the snake.

Near one point of the periphery of the nucleus of the red cell, a small bud appears. This tiny projection increases in size, and gradually becomes somewhat spherical. The bud thus formed is extruded and passes into the cytoplasm as a small, spherical body (figs. 8, 9). This is the Anaplasma. Sometimes two buds appear

side by side, and pass outwards attached to one another. On other, rarer occasions, more especially when the preparations have been examined under dark-ground illumination, a small stream of five or six buds is emitted from the nucleus, the result being a small rosette of Anaplasma in the cytoplasm of the host cell.

On a few rare occasions, the breaking up of large Anaplasma into smaller ones has been observed. The occurrence of more than one Anaplasma in a corpuscle (fig. 10) may be thus explained, or the bodies may have had separate origins. There is no special order or sequence about this disruption, which resembles the fragmentation seen in decaying nuclei of certain plant tissues. It is different from the true division of any protozoön nucleus with which I am acquainted.

The origin of the Anaplasma in mammalian blood has only been observed in a few instances. In such cases the host was a young animal and anaemic, and also showed a certain number of nucleated erythrocytes in its blood and in smears of organs made at death. Anaplasma-forms were found in preparations of the spleen, liver and bone-marrow, and similar bodies occurred free in the blood plasma. There was no difference in morphology, whatever the situation in which the structures were found.

I may mention that I have had the advantage of comparing my preparations with blood films containing Anaplasma, which films came from Theiler's laboratory in Pretoria.

#### THE NATURE OF ANAPLASMATA

The nature of the structures termed Anaplasma has been the subject of much controversy at different times, and very contradictory accounts of these bodies have been given by different workers. The earlier workers on piroplasmosis, for example, Smith and Kilborne (1893), noted small structures in the blood of cattle infected with Texas fever. They described them as round coccus-like bodies,  $0.2\mu$  to  $0.5\mu$  in diameter, which were usually situated at the periphery of the corpuscle. Some larger granules were also seen. These peripheral coccus-like bodies were considered to be probably the early stages of *Piroplasma bigeminum*. They noted that the peripheral bodies appeared in the blood as the number of blood corpuscles began to fall, that is, under conditions of anaemia.

The foregoing conception of the nature of coccus-like bodies, Anaplasmata or marginal points, continued in force till about 1910, when Theiler, working in South Africa, put forward the suggestion that the peripheral, coccus-like bodies of Smith and Kilborne were organisms independent of Piroplasmata and producing a different disease from the latter organisms. He considered the marginal points, as the structures had been often termed, to be the true excitants of gall-sickness in cattle, and created the genus *Anaplasma* for them, two species, *A. marginale* and *A. centrale* being subsequently differentiated.

Since Theiler's memoir appeared, a number of observations have been published that show the presence of Anaplasmata in many hosts, and in maladies not associated with gall-sickness or piroplasmosis. They have been found in young dogs and in marsupials in Australia. Donkeys suffering from trypanosomiasis in the Sudan show Anaplasmata, as do cats and rats suffering from the same disease. Guinea-pigs, monkeys, lemurs, calves, sheep, goats, horses, asses, pigs, rabbits, moles, mice, all have been reported as containing Anaplasmata, many of these hosts apparently being quite healthy. The bodies have been seen frequently in newly born animals. Certain authors, having observed coccus-like or Anaplasma-like bodies in the blood of animals experimentally infected with leishmaniasis, considered the Anaplasmata to be possibly the initial stages of the Leishman-Donovan bodies.

It is very interesting to note that Sangiorgi (1915) found Anaplasmata or marginal points in the splenic blood of a child from Catania who was infected with infantile kala-azar, a disease producing progressive anaemia. I, personally, have seen similar bodies in the blood corpuscles of children suffering from obscure forms of anaemia in England, and in the blood of a man returned from the Tropics, who had perhaps previously had malaria.

Anaplasmata probably have more than one origin, as is seen from the previous statements. Still another circumstance in which such bodies are found in the host vertebrate has been revealed by the work of Dias and Aragão (1913-14). These workers conducted a number of experiments on guinea-pigs, rabbits, dogs and cattle by inoculating them with phenylhydrazine, nitrobenzol, pyrogalllic acid, saponin, phosphorus in oil emulsion and trypan blue. Nitro-

benzol, pyrogallie acid and phenylhydrazine produced structures which were compared with preparations of natural Anaplasma obtained from Theiler. The staining reactions, size, morphology, lack of structure and pseudo-division forms were the same as those found in bovines naturally infected with anaplasmosis. The injection of haemolytic substances thus produced structures of the same nature as Anaplasma. The authors conclude that Anaplasma is not a protozoön, but is a product of the degeneration of the red cells due to the action of the haemolytic substances introduced into the host. The anaplasmosis of Brazil is considered to be really a piroplasmosis, in which the parasites remain chiefly in the internal organs of the host. The presence of Anaplasma in animals infected with worms is considered to be due to the haemolytic action of the toxins produced by the helminthes, while their occurrence in newly-born animals is ascribed to the activity of their haematopoietic organs. Laveran and Franchini (1914) have confirmed the production of Anaplasma-like bodies by the use of phenylhydrazine.

Early in 1915, a paper by Veglia appeared containing an account of the results obtained by him in culturing *Anaplasma marginale*, using various media. He found that the number of corpuscles showing Anaplasma increased greatly in the cultures. At lower temperatures, the round form predominated. Later, the parasites became larger and assumed a somewhat triangular or quadrangular shape. Schizogony into three, four and eight is believed by him to occur. Diplococcoid forms were also observed. A series of figures showing the great increase in the numbers of infected corpuscles is given. Regarding both the increase in numbers of the Anaplasma and of the infected corpuscles, I would suggest that another interpretation may be given. It seems to me that in these cases haemolytic substances are produced in the culture medium from chemical changes in some of the red corpuscles. The quantity of such substances increases with the progress of the experiment, and acting on the remaining red corpuscles, a number of Anaplasma are produced; the numbers of infected erythrocytes would, consequently, increase for some time. As there may have been an increase in the quantity of haemolytic substances in the blood of an animal suffering from masked piroplasmosis, Veglia's conclusion that the progress of his

cultures was on the same lines as that of natural anaplasmosis is explicable.

It must be mentioned that Franchini and Mantovani (1915) found in a culture of rat's blood on Novy's medium mixed with glucose, a number of small bodies presenting the appearance of *Anaplasma* in stained preparations. It is possible that the presence of haemolytic substances in the culture resulted in the production of these bodies.

It is interesting to note with regard to the origin of some Anaplasmata, that over twenty years ago, Smith and Kilborne, with remarkable foresight, suggested that some of the bodies now termed *Anaplasma* were 'probably remnants of the nucleus of the ancestor of the [red] corpuscle—the haematoblast.'

My own experiences suggest that Anaplasmata may be of nuclear origin, and may be the results of anaemia induced in the host by such diseases as herpetomoniasis.

If, however, *Anaplasma* be considered organismal, it affords an interesting example of what is, perhaps, a phylogenetic and recapitulative type of primitive Protozoön. *Anaplasma* might also represent an organism which has been secondarily reduced in size and structure.

### SUMMARY

Anaplasmata may occur in healthy and in anaemic vertebrate blood. The structures, also called marginal points and peripheral coccus-like bodies, are probably of diverse origin. It is doubtful if they are organismal in nature.

Anaplasmata have been found by me in warm and cold-blooded vertebrates, wherein conditions such as herpetomoniasis and anaemia occurred. Some of the bodies originate from the nucleus of the erythrocyte or erythroblast, under the influence of haemolysis.

The *Anaplasma*-like bodies were basophilic, apparently composed of chromatin or of a substance giving a similar staining reaction, and were homogeneous in structure. They varied from  $0.3\mu$  to  $2.0\mu$  in diameter, often being about  $0.5\mu$ . Binary and multiple forms, which might be interpreted as phases of division, were seen.

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